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Circadian Neurotransmitter Activity Regulating Seasonal Conditions in Three Avian Species.

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CIRCADIAN NEUROTRANSMITTER ACTIVITY REGULATING SEASONAL CONDITIONS IN THREE AVIAN SPECIES

A Dissertation

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by
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ABSTRACT

Seasonal reproductive and migratory conditions in the migratory white-throated sparrow, Zonotrichia albicollis, may be regulated by two circadian neural oscillations. The results of experiments presented within suggest that serotonergic and catecholaminergic neurotransmitter activities are expressions of the oscillations. Serotonergic activity appears to maintain the daily plasma corticosterone rhythm which, in turn, entrains daily rhythms of tissue sensitivity. A single injection of 5-HTP (serotonin precursor) raised plasma corticosterone concentrations in Japanese quail, Coturnix c. japonica, and white-throated sparrows after one to three hours. In quail, 5-HTP injections induced gonadal growth when given at 12 h before the onset of a 6-h daily photoperiod but had no stimulatory effect at three other temporal relations. 5-HTP and corticosterone similarly induced gonadal growth in house sparrows, Passer domesticus, when injected 12 h before the onset of a 9-h photoperiod.

In white-throated sparrows maintained in continuous light, daily injections of DOPA (catecholamine precursor) at 12 h after 5-HTP stimulated gonadal growth and fattening, conditions associated with the spring migratory period. An 8-h injection interval inhibited gonadal growth and fattening, conditions found in birds during late summer. A

3-h relation inhibited gonadal growth and possibly stimulated fattening, conditions associated with the fall migratory period. In house sparrows, daily injections of DOPA 16 h after 5-HTP depressed gonadal development whereas an 8-h interval maintained oviducts near the photostimulated, pre-experimental levels. In previous studies, similar results were obtained with daily injections of corticosterone and prolactin in the same hourly relations in both species. Catecholaminergic activity (possibly dopamine) appears to be part of the feedback mechanism which regulates the daily prolactin rhythm.

The temporal synergism of neurotransmitter activity apparently stimulates the seasonal changes between two circadian neural oscillations. Daily injections of DOPA 12 h after 5-HTP for 14 days may reset the circannual clock in photorefractory white-throated sparrows during the fall so that their annual cycle is reset into the spring condition. This temporal relation apparently comprises the circannual mechanism that controls the orderly seasonal changes in reproductive and migratory events.

GENERAL INTRODUCTION

Most bird species of the temperate zone have a distinct annual cycle of physiological and behavioral events. These include annual cycles of gonadal growth, reproductive behavior, molt, fattening, and for many species, migratory activity. The studies by Rowan (1929, 1930, 1932) with crows and juncos first established that the increasing photoperiod is the principal cue for the induction of spring conditions. Since that time photoperiodism has been demonstrated in many species of temperate zone birds (see reviews, Farner, 1964; Gwinner, 1975).

Bünning (1936, 1960) proposed that circadian rhythms are involved in photoperiodism. Briefly stated, some aspect of the photoperiod (ie. light onset) entrains an endogenous rhythm of photosensitivity. The first half of the cycle is light-requiring (photophil), the second half is dark-requiring (scotophil). Bünning proposed that photoperiodic induction occurs when long days cause light to extend into the scotophil half of the cycle or when light is coincident with a sensitive phase of a circadian rhythm. Because photoperiodic induction depends upon the coincidence of light with the light-sensitive phase of a photosensitivity rhythm, Bünning's hypothesis has been termed an 'external coincidence' model. Strong support for this hypothesis in birds was found in white-throated

sparrows, Zonotrichia albicollis (Jenner and Engels, 1952; Meier, 1976), house finches, Carpodacus mexicanus (Hammer, 1963; 1966), house sparrows, Passer domesticus (Menaker and Eskin, 1967), Japanese quail, Coturnix c. japonica (Follett and Sharp, 1969), and white-crowned, Zonotrichia leucophrys, and golden-crowned, Z. atricapilla, sparrows (Turek, 1972).

However, the neural and endocrine components of the photoperiodic response are only beginning to be understood. Most studies concerning the neuroendocrine control of avian breeding cycles have attempted to delineate the neural pathways and neurotransmitters involved in the release of gonadotropic hormones. Much effort has been spent in identifying hypothalamic aminergic pathways (Follett and Davies, 1975) and isolating monoamines which supposedly stimulate or inhibit gonadotropin release (Davies and Follett, 1974; Campbell and Wolfson, 1974; El Halawani and Burke, 1975). In spite of considerable evidence for the involvement of circadian rhythms in photoperiodism, few studies have attempted to incorporate this information into a model of neuroendocrine regulation in birds.

An extension of Bünning's hypothesis for photoperiodism has been termed an 'internal coincidence' model because it involves two circadian oscillators. The photoperiodic response would depend on the alignment of the two

oscillators in a certain phase relationship (see review by Gwinner, 1975). For several years Meier and colleagues have accumulated data which support such a model (see review by Meier and Ferrell, 1978). Daily rhythms of adrenal cortical hormone (ACH) and prolactin are believed to be expressions of the two oscillators. Studies from a variety of vertebrates (see Meier, 1975) suggest that one oscillator entrains a daily rhythm of ACH which, in turn, sets daily rhythms of tissue sensitivity. The second oscillator is expressed as a daily rhythm of prolactin release. Thus, stimulation occurs when the daily prolactin peak coincides with a period of tissue sensitivity.

Other evidence suggests that the same circadian oscillators are involved in the control of the annual cycle. The phases of the daily rhythms of both corticosterone and prolactin apparently shift throughout the year in the white-throated sparrow (Meier et al., 1969; Dusseau and Meier, 1971; Meier and Fivizzani, 1975). Apparently, the two circadian oscillators vary in phase with respect to one another throughout the year. The daily pattern of endogenous corticosterone and prolactin release varies throughout the year in white-throated sparrows; that is, in the late spring, a 12-h interval occurs between the daily rise of plasma corticosterone and a daily release of pituitary prolactin whereas in the summer the interval is approximately 6 - 8 hours. Daily injections of corticosterone

and prolactin in either a 12 or 8-hour interval induced spring or summer-like conditions, respectively. Seasonal conditions depend upon the coincidence of some phase of the daily prolactin rhythm (expression of second oscillator) with a sensitive phase of an entraining ACH rhythm (expression of first oscillator).

Thus an internal coincidence model that incorporates circadian hormone rhythms may account for all phases of the annual cycle in white-throated sparrows. The metabolic and behavioral events are closely coordinated so that the bird is physiologically prepared for the demands of reproductive or migratory activity. Induction of the entire repertoire of seasonal conditions with timed corticosterone and prolactin injections suggests that these hormones influence the basic neural regulatory centers which control the annual sequence. Therefore, timed injections of corticosterone and prolactin may set the phase of a circannual mechanism by way of their influence on the activity of central neurons and their neurotransmitters.

Three classes of neurons that have been widely implicated in hormone regulation are those which secrete 5-hydroxytryptamine (serotonin) and the catecholamines-dopamine and noradrenaline. The literature, as reviewed later, suggests that the daily adrenal corticoid rhythm is influenced by a rhythm of serotonergic activity. Regulation of the daily prolactin rhythm however, may be by way

of dopaminergic activity. These tentative conclusions are based, in part, on studies in which drugs (precursors, synthesis blockers, etc.) that affect serotonergic and catecholaminergic activity altered endogenous adrenal corticoid or prolactin levels.

Therefore, timed injections of appropriate neurotransmitter-affecting drugs may induce seasonal conditions in a fashion similar to conditions produced by timed hormone injections. The experiments in Section I examined the ability of 5-HTP (serotonin precursor) to alter the plasma corticosterone concentration. Because corticosterone is thought to entrain circadian rhythms of photosensitivity, a circadian rhythm of gonadal responsiveness to drug injections may occur as well. Therefore, in Section II daily drug injections were given to birds at specific times of a short-day photoperiod to test for possible variations in gonadal development. Inasmuch as a daily rhythm of fattening and gonadal response occurs with timed, daily injections of corticosterone and prolactin, neurotransmitter-affecting drug injections may elicit similar responses. Accordingly, the experiments in Section III examined the possibility that a temporal synergism between serotonergic and catecholaminergic activity might influence gonadal development and fattening. In sparrows held on a constant light regimen to remove the influence of photoperiodic cues, injections of neurotransmitter precursors

were given daily at specific hourly intervals. Because daily injections of corticosterone and prolactin reset the circannual mechanism in photorefractory white-throated sparrows (Meier et al., in press), I reasoned that appropriate drug injections in a similar timed sequence may also reset the circannual cyle into a spring condition. Therefore, in the experiments in Section IV, daily injections of serotonin- and catecholamine-affecting drugs were given in an hourly pattern believed to mimic the endogenous temporal pattern of neurotransmitter activity thought to participate in the regulation of spring migratory conditions.

SECTION I

SEROTONERGIC STIMULATION OF PLASMA CORTICOSTERONE IN JAPANESE QUAIL, *Coturnix coturnix japonica* AND WHITE-THROATED SPARROWS, *Zonotrichia albicollis*

INTRODUCTION

Among avian species, daily rhythms of corticosterone (CS) concentration have been found in Japanese quail (Boissin and Assenmacher, 1968; 1970), white-throated sparrows (Dusseau and Meier, 1971; Meier and Fivizanni, 1975), ducks (Assenmacher and Boissin, 1972), common pigeons (Joseph and Meier, 1973; Sato and George, 1973) and chickens (Majsa et al., 1976). The importance of a CS rhythm for birds rests in part on its postulated roles in both photoperiodism and regulation of the annual cycle (General Introduction). However, the identity of the neural system which mediates environmental entrainment of the CS rhythm is not well understood in birds. Serotonin-secreting neurons projecting to the forebrain may modulate the rhythmic release of corticosterone. In Japanese quail, para-chlorophenylalanine (PCPA), an inhibitor of 5-HTP (serotonin precursor) synthesis, dampened the plasma CS rhythm (Boissin and Assenmacher, 1971). In mammals, pharmacological disruption of serotonin synthesis also altered the normal circadian rhythm of plasma corticosterone (Krieger and Rizzo, 1969; Scapagnini et al., 1971). Although some evidence from mammalian studies suggests that noradrenergic stimulation raises plasma CS levels (Naumenko, 1968; Krieger and Krieger, 1970), most workers have ascribed an inhibitory role for catecholaminergic activity in

mammalian plasma CS regulation (review: Fuxe et al., 1970).

Because of the importance of the daily CS rhythm in avian photoperiodism and circannual mechanisms, further study of the neurotransmitter - plasma CS relationship seemed warranted. Systemic injections appeared to be the most expedient method of stimulating neurotransmitter activity. However, injections of neurotransmitters themselves are generally not effective in raising central activity levels of these substances because they do not readily cross the blood - brain barrier. Systemic injections of precursors do cross into the central circulation where they are taken up by the appropriate neurons and converted into neurotransmitter molecules. A single systemic injection of 5-hydroxytryptophan (5-HTP, serotonin precursor) in rats raised brain serotonin levels after one hour, and the levels remained elevated four hours after injection (Ternaux et al., 1975). Likewise, systemic injection of the catecholamine precursor dihydroxyphenylalanine (DOPA) increases the activity of brain catecholaminergic neurotransmitters in rats. Ten minutes after intravenous injection of ^{14}C - L-DOPA, 67 percent of the brain radioactivity was dopamine and its metabolites, including noradrenaline (Shindo et al., 1973). Therefore, systemic injections of these neurotransmitter precursors cross into the central circulation and are rapidly converted into the

neurotransmitters. Accordingly, this method of stimulating brain neurotransmitter activity was used to test neural regulation of the plasma corticosterone levels in quail and white-throated sparrows.

MATERIALS AND METHODS

Experiment 1. The female Japanese quail, Coturnix coturnix japonica, were reproductively active adults on LD 16:8 (light onset: 0900). Beginning at 0900 hrs. on 12 January 1979, blood samples were drawn by wing-vein puncture from 12 birds. Immediately after blood sampling, a particular bird was injected subcutaneously (sc) with either 0.1 ml saline (0.9%) or 1 mg of fluoxetine (Lilly 110140) suspended in 0.1 ml saline. Six birds received fluoxetine treatment (a serotonin reuptake inhibitor, see Appendix I) and six were injected with saline. Pre-injection sampling and injections were completed by 0915. At 1000, each fluoxetine-treated bird was injected with 10 mg 5-HTP suspended in saline. At 1200, blood samples were again taken in all birds.

Experiment 2. A similar experiment was performed on 5 July 1979 with reproductively active quail. Photoperiodic conditions and blood sampling-injection procedures were similar to those of the first experiment. At light onset (0900), blood samples were taken immediately prior to a single injection of either saline, 5-HTP, or DOPA. There were six birds in each of these three groups. One hour later, blood samples were taken again according to the pre-injection sampling sequence.

Experiment 3. The effect of 5-HTP injections upon plasma CS concentrations was also tested in adult female white-throated sparrows, Zonotrichia albicollis. On 4 April 1979, birds were removed from an outdoor aviary and placed two to a cage (16 x 22 x 22 cm) on a light regimen which approximated the natural photoperiod. At this time of year, this species is photosensitive, but not photostimulated by short daylengths. On 6 April, pre-injection blood samples were taken starting at 2030, a time when plasma corticosteroid levels are believed to be low in this species in April (Meier and Fivizzani, 1975). Twelve birds were injected with 0.05 ml of 0.9% saline and 12 with 2 mg 5-HTP suspended in 0.05 ml saline. An injection was given immediately following each individual sampling. One hour after injections six birds from each treatment group were sampled again. Blood samples were taken from the remaining birds three hours following injections.

Assay Procedure. Plasma was separated immediately from all blood samples by centrifugation and stored at -22^{o} until assayed for CS concentration. Plasma CS concentration was assayed by a modification of the competitive protein binding procedure (Murphy, 1967). Duplicate 10 ul samples were extracted with 1.0 ml absolute ethanol, and the ethanol extracts were dried under air at 45^{o}. Four sets of tubes which contained 0.0, 0.5, 0.7, and 1.0 ng CS in absolute ethanol were run in triplicate and also dried for preparation

of a standard curve. One ml of corticosterone-binding-globulin solution, containing 1.0% male human plasma and 0.04 uCi tritiated CS (47.5 Ci/mmol) in distilled water, was added to all assay tubes. The tubes were shaken for 15 seconds with a vortex stirrer and then incubated in a 45^{o} water bath, shaken for 15 seconds, and transferred to a 4^{o} water bath for 30 minutes. At the end of this cold incubation, 30 mg Florisil was added to each tube and the tubes were shaken for 30 seconds. After the Florisil had settled, a 0.5 ml aliquot was removed from each tube and added to 5.0 ml liquid scintillation fluid containing 0.4% p-terphenyl and 33% Triton X - 100 in spectrograde toluene.

The samples were counted to a 98% degree of accuracy on a Beckman LS - 8000, and the mean counts per minute (cpm) for replicates were expressed as a percent of the cpm in the zero tubes of the standard curve. A standard curve of percent of zero value vs. ng CS was used to estimate the amount of CS in the samples, and plasma CS concentrations were expressed as ug per 100 ml plasma (ug %). The extraction efficiency was approximately 100%. The intraassay variation was 3% and the interassay variation was 5%.

RESULTS

Experiment 1. The mean plasma CS concentration of quail sampled before fluoxetine and 5-HTP injection was 1.6 ± 0.3 ug %. Two hours after the 5-HTP and three hours after fluoxetine injections, the CS levels rose to 4.1 ± 0.6 ug %, a significant ($p < .01$, paired t-test) increase over pre-injection levels (fig. 1A). Plasma CS concentration decreased slightly, but not significantly in a saline-injected group during the same time period.

Experiment 2. The mean CS concentration of female quail before 5-HTP injection was 2.5 ± 0.4 ug %. One hour after sampling and injection, levels had risen ($p = .05$) to 4.1 ± 0.9 ug % (fig. 1B). DOPA injections reduced (p .02) plasma CS levels to 2.3 ± 0.3 ug % compared with pre-injection levels (3.3 ± 0.3 ug %) whereas saline injections had no effect after one hour.

Experiment 3. A single injection of 5-HTP to white-throated sparrows raised ($P = .01$) plasma CS concentrations after three hours (5.6 ± 0.6 ug %) compared with pre-injection levels (3.3 ± 0.5 ug %) (fig. 2). Saline injections caused no significant change in CS levels at either one or three hours post-injection. A slight but non-significant rise occurred one hour after 5-HTP treatment.

Figure 1. Effect of serotonergic or catecholaminergic - potentiating drugs on plasma corticosteroid concentration in photostimulated female Japanese quail, Coturnix coturnix japonica (LD 16:8). Blood samples were drawn at light onset (0900) from each bird at the time of injection (shaded bars) and at a specified interval later. (A) Exp. 1. Birds injected with fluoxetine (1 mg) at 0900, 5-HTP (10 mg) at 1000 and sampled again at 1200. (B) Exp. 2. Birds injected with 5-HTP, DOPA (24 mg) or saline (0.9%) and sampled again one hour later. Six birds per group.

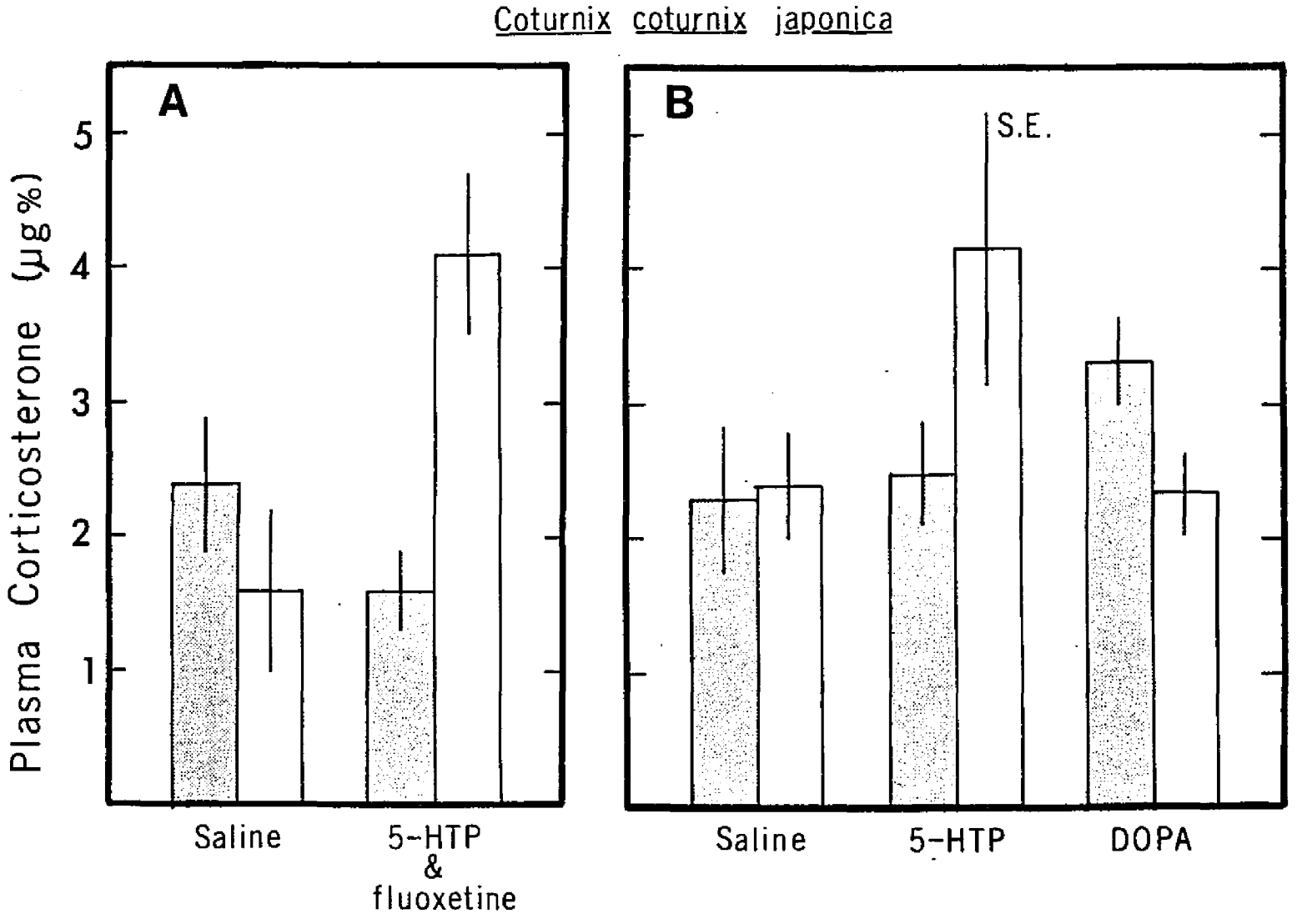
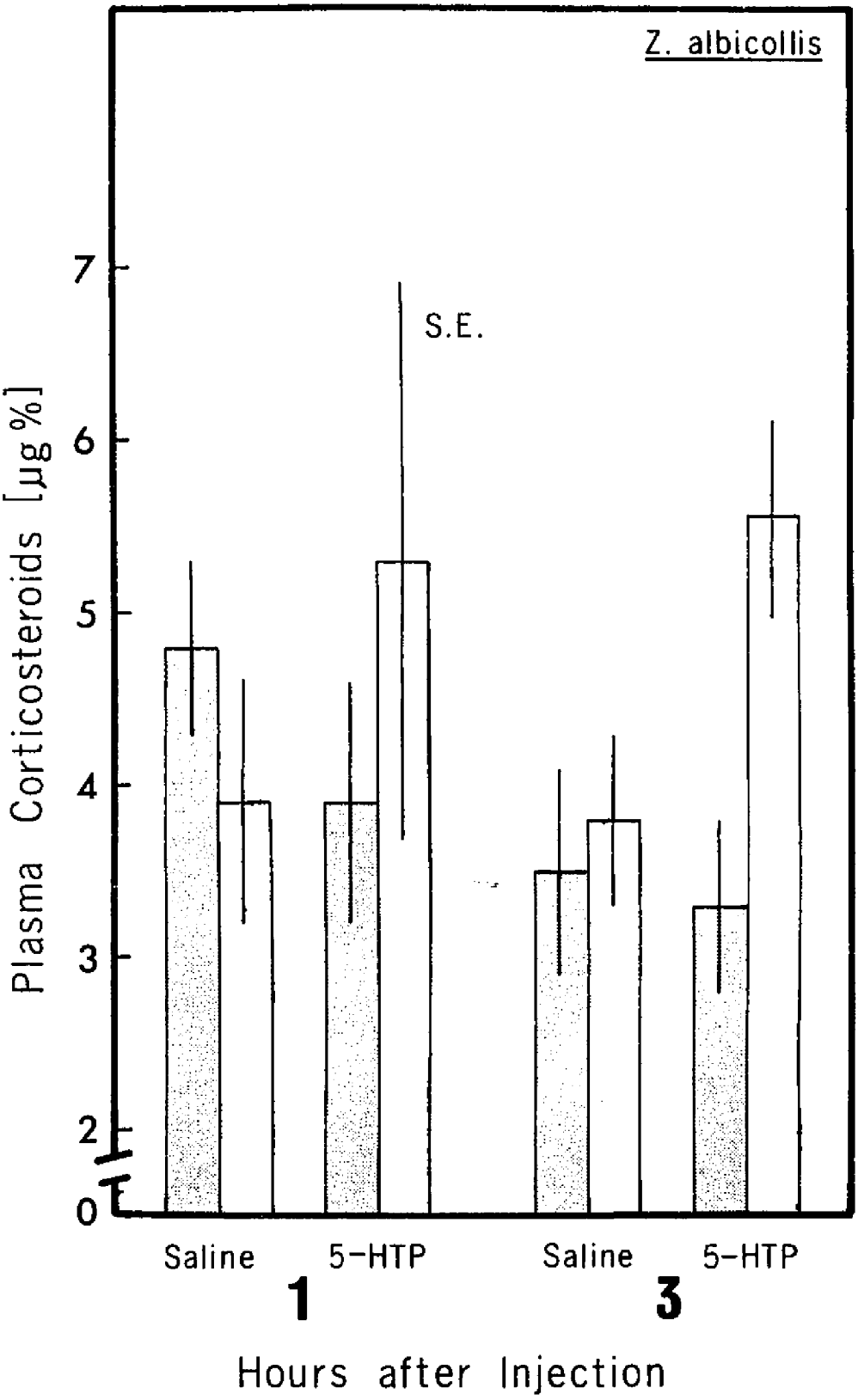

Coturnix coturnix japonica
A
B
S.E.
Plasma Corticosterone (μg%)
5
4
3
2
1
Saline
5-HTP
&
fluoxetine
Saline
5-HTP
DOPA

Figure 2. Effect of a single 5-HTP injection (2 mg) on plasma corticosteroid concentration in female white-throated sparrows, Zonotrichia albicollis. Light period: 0530 to 1730. Blood samples were drawn at 0 (shaded bars), 1 and 3 hours after 5-HTP or saline injections. Six birds per group.


Z. albicollis
S.E.
Plasma Corticosteroids [µg %]
7
6
5
4
3
2
0
Saline
5-HTP
Saline
5-HTP
1
3
Hours after Injection

DISCUSSION

This study indicates that 5-HTP injections raise plasma corticosteroid (CS) levels in quail and white-throated sparrows. Hormone concentrations in quail one hour after injections (4.1 $\pm$ 0.9 ug %, Fig. 1B) are equivalent to the daily peak concentrations reported for untreated quail (Boissin and Assenmacher, 1968). Likewise, 5-HTP injections to white-throated sparrows raised plasma CS concentrations (5.6 $\pm$ 0.6 ug %) to the peak daily levels when measured for birds in a similar phase of their annual cycle (Meier and Fivizzani, 1975). Thus, drug-induced serotonergic activity raises plasma CS to levels which approximate the endogenous peak daily concentrations that occur in both species. Other evidence suggests that a daily rhythm of serotonergic activity is correlated with the daily plasma CS rhythm. In quail, PCPA (5-HTP synthesis inhibitor) injections dampened the daily plasma CS rhythm (Boissin and Assenmacher, 1971).

In rats, systemic 5-HTP injections elevated brain 5-HT levels for four hours or longer (Korf et al., 1974; Ternaux et al., 1974; Saavedra, 1975; Warsh and Stancer, 1976). Thus, prolonged serotonergic activity could account for a subsequent period of sustained plasma CS levels (at least three hours) observed in white-throated sparrows and quail. Fluoxetine pretreatment appeared to augment the rise

in CS levels after 5-HTP injections in the quail. Fluoxetine stimulates synaptic serotonergic activity by inhibiting serotonin's re-uptake into axon terminals. My results are similar to those in which fluoxetine potentiated CS elevations induced by 5-HTP injections in rats (Fuller et al., 1976) and mice (Meyer et al., 1978). Catecholaminergic stimulation appears to inhibit adrenal cortical activity in quail. Systemic injections of DOPA significantly depressed plasma CS concentrations. DOPA injections also depressed plasma CS levels in rodents (Ganong, 1972; Scapagnini et al., 1972).

Therefore, the daily plasma CS rhythm may be regulated by the opposing actions of daily serotonergic and catecholaminergic activities. While serotonergic activity stimulates plasma CS levels, the elevated hormone levels may, in turn, exert a temporary positive feedback on serotonergic activity. In rats, midbrain tryptophan hydroxylase activity (rate-limiting enzyme in serotonin synthesis from tryptophan) increased four hours after a single, systemic corticosterone injection (Azmitia and McEwen, 1969). Likewise, in rats, corticosterone increased the rate (30 minutes) of conversion of ^{14}C - tryptophan to ^{14}C - serotonin (Millard et al., 1972). Conversely, the reduction of plasma CS levels by DOPA injections suggests that troughs in the daily CS rhythm are due to periods of increased catecholaminergic activity.

A daily rhythm of plasma CS concentration controls many other physiological rhythms in birds (see Meier and Ferrell, 1978). Serotonergic activity, via its influence on plasma CS levels, may participate in the entraining aspect of these rhythms. Reproductive activity is stimulated when quail and white-throated sparrows are exposed to long-day photoperiods. Circadian rhythms are thought to be involved in this photoperiodic response. One hypothesis ascribes a role for a daily corticosteroid rhythm as an entrainer for circadian rhythms involved in photoperiodism (see General Introduction). If serotonergic activity maintains the corticosteroid rhythm as proposed, then a circadian rhythm of photosensitivity may be entrained by serotonergic activity. Timed serotonergic activity may be expected to stimulate reproductive development only at certain times relative to a short photoperiod. The experiments in the following section examine further the possibility that serotonergic activity may be involved in photoperiodism and in the regulation of the annual cycle in white-throated sparrows.

SECTION II

NEUROTRANSMITTER ACTIVITY AND PHOTOPERIODISM IN JAPANESE QUAIL, WHITE-THROATED SPARROWS AND HOUSE SPARROWS, *Passer domesticus*

INTRODUCTION

Rowan (1929, 1930, 1932) first demonstrated that the major determinant for the onset of reproductive and spring migratory activity in temperate zone birds is the increasing daylenth in spring. Bünning (1936, 1960) proposed that circadian rhythms are involved in the photoperiodic response in many plants and animals. The daily photoperiod is thought to entrain a circadian rhythm of photosensitivity; the photoinducible phase of this rhythm occurs 12-24 hours after light onset. If light occurs during the photoinducible phase (as when days are long during late spring and summer months), then photoinduction of gonadal growth occurs. Considerable support for Bünning's model now exists for several avian species (see General Introduction).

As described previously, an internal coincidence model has also been proposed as an extension of Bünning's hypothesis. A daily rhythm of plasma adrenal cortical hormone concentration is a part of this model and is thought to be an expression of the same neural oscillation which entrains a daily rhythm of photosensitivity (Meier and MacGregor, 1972; Meier and Dusseau, 1973). In white-throated sparrows maintained on a nonstimulatory daily photoperiod (LD 6:18), daily injections of corticosterone elicited increases in testicular and ovarian weights when the injections were made 18 h before the onset of light and not when the injections were made at 6 or 12 h beforehand (Meier and Dusseau, 1973).

A number of studies (see Section I) indicate that brain serotonergic activity is involved in the maintenance of the plasma corticosterone rhythm in birds and mammals. If the corticosterone rhythm and the photosensitivity rhythm are expressions of the same oscillation, perhaps stimulation of timed serotonergic activity can entrain both rhythms. Injections of 5-HTP (serotonin precursor) given at a specific time during an L:D cycle may mimic the effects of corticosterone injections which stimulate gonadal growth only at a certain time during a nonstimulatory photoperiod regime. Reproductively inactive (as judged by the regressed testicular condition) Japanese quail (Coturnix c. japonica) were used in Experiment 1 to test this hypothesis since a daily rhythm of plasma corticosterone is known for this species (Boissin and Assenmacher, 1968) and because their reproductive system is particularly sensitive to photoperiodic manipulation (Lofts et al., 1970).

Corticosterone injections stimulated testis growth in house sparrows when given 6 h, but not 18 h, before light onset (LD 6:42) (Meier and Dusseau, 1973). The results indicate that corticosterone injections entrained a rhythm of photosensitivity such that a light interval 6-12 h after injections occurred during a photoinducible phase. If neurotransmitter activity can also entrain this rhythm, I predicted that drug-induced stimulation of that activity approximately 12 h before the onset of a nonstimulatory

photoperiod would stimulate gonadal growth. Therefore, in Experiment 2, gonadal development was examined in house sparrows after a period of daily drug or corticosterone injections given 12 h before the onset of a 9 h photoperiod.

According to Bunning's model for photoperiodism, coincidence of light with a photoinducible phase initiates gonadal development. Interrupted-night photoperiod regimes have been cited as evidence in support of Bunning's model (see Gwinner, 1975). In general, a nonstimulatory cycle (ie., LD 6:18) is interrupted by short intervals (15 minutes - 2 hours) of light at different times during the dark. If the second light interval coincides with the photoinducible phase entrained by the initial photoperiod, gonadal growth occurs. Increased catecholaminergic activity occurred during the photoinducible phase in white-crowned sparrows (Zonotrichia leucophrys gambelii) (Warren et al., 1973). Photoperiodic stimulation of gonadal growth may also involve decreased serotonergic activity (Calas, 1975; El Halawani et al., 1978). Drugs that alter neurotransmitter activity may affect reproductive development if given during the photoinducible phase. I reasoned that gonadal growth which occurs in birds on an interrupted-night photoperiod regime may be inhibited by timed drug injections given prior to the second light interval. Therefore, in Experiment 3, white-throated sparrows were maintained on

an interrupted-night regime and injected daily with serotonergic-stimulating and catecholaminergic-inhibiting drugs near the predicted photoinducible phase.

MATERIALS AND METHODS

Three experiments were performed with photosensitive, unstimulated Japanese quail, white-throated sparrows, and house sparrows. The quail (Coturnix c. japonica) were kindly provided by the Poultry Department at LSU. The white-throated sparrows (Zonotrichia albicollis) were collected from wintering flocks near the LSU campus. The house sparrows (Passer domesticus) were also collected locally shortly before the experimental period.

Experiment 1. Adult male quail were maintained on a 6-h daily photoperiod (LD 6:18) beginning in March 1979. Testicular regression was complete after six weeks and 14 daily injections of either saline or 5-HTP (10 mg) were then given at 0, 6, 12, or 18 hours after light onset (0900). There were five birds in each of the eight groups. Testicular size was determined by unilateral laparotomy before and after experimental treatment. A small incision was made between the last two left ribs. Calipers were used to measure length (L) and width (W) of the left testis to the nearest 0.1 mm. The measurements were converted into volume (mm^3) by using the formula for an ellipsoid: $4/3\ (W/2)^2\ (L/2)$. Volume measurements in cubic mm correlate well with single testis weights (mg) when both measurements have been taken in a particular bird.

Experiment 2. A previous study with house sparrows indicated that corticosterone injections stimulated gonadal growth when a light period occurred 6-12 h following injections but not 18-24 h afterwards (Meier and Dusseau, 1973). Accordingly, injections in the present experiment were made only at 12 h before light onset (LD 9:15) to test this photoperiodic response further in house sparrows with corticosterone and neurotransmitter-affecting drugs.

Male house sparrows were removed from an outdoor aviary on 17 January 1979 and housed individually in small cages on LD 9:15. Four injection groups were formed with five to seven birds per group. Birds received single daily injections of either 5-HTP (2 mg), corticosterone (25 ug), DOPA (4 mg), or saline 12 h after light onset (0900) for six weeks beginning 19 January. Testicular size was determined biweekly by laparotomy.

Experiment 3. Twelve male white-throated sparrows were transferred during January 1978 from an outdoor aviary to cages indoors where they were kept on LD 6:18. Unilateral laparotomies were performed on each bird at the time of transfer. Beginning 15 January, a 2-h light interruption was given during the dark beginning 15 h after the onset of the 6-h photoperiod and drug injections were started.

The literature suggests that serotonergic activity serves in two capacities in the photoperiodic response. In one role, serotonergic activity may be an expression of the

daily photosensitivity rhythm. As such, it may entrain a rhythm of tissue sensitivity via its positive feedback on the daily corticosteroid rhythm (see Section I). I reasoned that 5-HTP injections given at 0-h (onset of 6-h photoperiod) would augment entrainment of the photosensitivity rhythm at that hour. In a second capacity, serotonergic activity during the photoinducible phase may inhibit gonadal development. Therefore, fluoxetine (inhibitor of serotonin re-uptake) may inhibit gonadal growth if injected during the time when the coincidence of light with the photoinducible phase would normally stimulate gonadal growth.

Accordingly, one injection group received a daily injection (sc) regimen as follows: 5-HTP (2 mg) at 0-h (onset of 6-h photoperiod) followed in 12 h by α-methyl-para-tyrosine (α-MT, catecholamine synthesis inhibitor, Appendix 1) and in 15 h by fluoxetine (0.25 mg). Thus fluoxetine was given at the onset of the 2-h light interruption and α-MT was given 3 h prior to that time. A second group recevied daily injections of 5-HTP at 0-h followed in 12 and 15 h by saline injections. Injections were given for 13 days after which each bird was examined again by laparotomy.

RESULTS

Experiment 1. This experiment was performed to determine whether 5-HTP injections at any of four times relative to a 6-h daily photoperiod might stimulate gonadal growth in quail. No significant change in testis volume occurred in any of the quail injected for 2 weeks with saline at any time or with 5-HTP at 0, 6, or 18 hours after light onset (LD 6:18). The mean testes volumes after treatment ranged from 7.4 ± 1.0 mm^3 to 11.2 ± 4.5 mm^3. However, substantial testis growth occurred in 2 of 5 birds injected with 5-HTP at 12 hours after light onset (fig. 3). Because 3 birds were totally unresponsive to 5-HTP at 12 hours, individual testis volumes ranged from 7.1 to 110.0 mm^3 after treatment and the mean post-injection testis volume (38.8 ± 19.8 mm^3) is not significantly different ($p = .11$, t-test) from pre-injection levels (13.5 ± 2.5 mm^3). The mean testis volume of the two responsive birds was 82.1 ± 28.0 mm^3.

Experiment 2. In a previous study, gonadal growth occurred in house sparrows exposed to light 6-12 h after a daily injection of corticosterone (Meier and Dusseau, 1973). In this experiment, birds were exposed to a light period 12-21 h (LD 9:15) after a daily injection of corticosterone, 5-HTP, DOPA, or saline. Testes were completely regressed (mean testis volume, 10 birds = 1.5 ± 0.1 mm^3) prior to the start of the injections. After two weeks of injections, the testes

of individuals in certain groups had increased in size, whereas those in other groups had not. No change occurred in birds that received either saline or DOPA injections 12 hours after onset of a 9-h photoperiod (LD 9:15). However, small but noticeable increases in testis volumes were evident in some individuals of the 5-HTP and corticosterone groups (fig. 4). In order to elucidate the effects of these injections, the experiment was continued for another four weeks. At the end of that period, testis growth was observed in 3 of 5 corticosterone-injected birds and in 3 of 7 5-HTP - injected birds. No significant changes were observed in any DOPA or saline-injected bird. After six weeks, mean testis volume was greatest in the corticosterone group. However, because two birds were unresponsive to treatment (range of testis volumes: 1.8 to 26.8 mm^3), the group mean (10.1 $\pm$ 4.6 mm^3) is only marginally different ($p = .06$, Student's *t*-test) from that of saline-injected birds (2.0 $\pm$ 0.2 mm^3). When the three birds responsive to corticosterone injections are considered alone (mean $\pm$ S.E.: 15.3 $\pm$ 6.0 mm^3), the difference from the saline group is evident ($p = .01$). A small, but statistically significant ($p = .02$), *t*-test) increase in mean testis volume (3.8 $\pm$ 0.7 mm^3) occurred in the 5-HTP - injected group.

Experiment 3. Interrupted-night experiments have been used to identify the time of the photoinducible phase of the photosensitivity rhythm in several avian species (see Introduction). In order to study this photoperiodic response further, daily injections of monoamine-affecting drugs were given to white-throated sparrows exposed to an interrupted-night photoperiod regime.

Testes of all white-throated sparrows were regressed (mean testis volume, 12 birds = 1.5 ± 0.2 mm^3) prior to exposure to the skeletal light period and the start of injections. The mean left testis volumes increased ($p = .02$, paired t-test) to 5.6 ± 1.4 mm^3 in group 1 which received daily injections of 5-HTP at light onset (0-h) and saline injections at 12 and 15 hours (fig. 5). However, no significant change in testis size occurred (mean volume: 1.7 ± 0.2 mm^3) in group 2 which were injected with 5-HTP at 0-h, and α-MT and fluoxetine at 12 and 15 hours respectively.

Figure 3. Testis growth in Japanese quail after treatment with 5-hydroxytryptophan (5-HTP) or saline at one of four different times of day (LD 6:18) for 14 days. Left testis volume was determined for each bird (5 birds per group) before (shaded bars) and after treatment (open bars). Mean testis growth in 2 of 5 birds (dotted extension) is shown for the 12-h group.

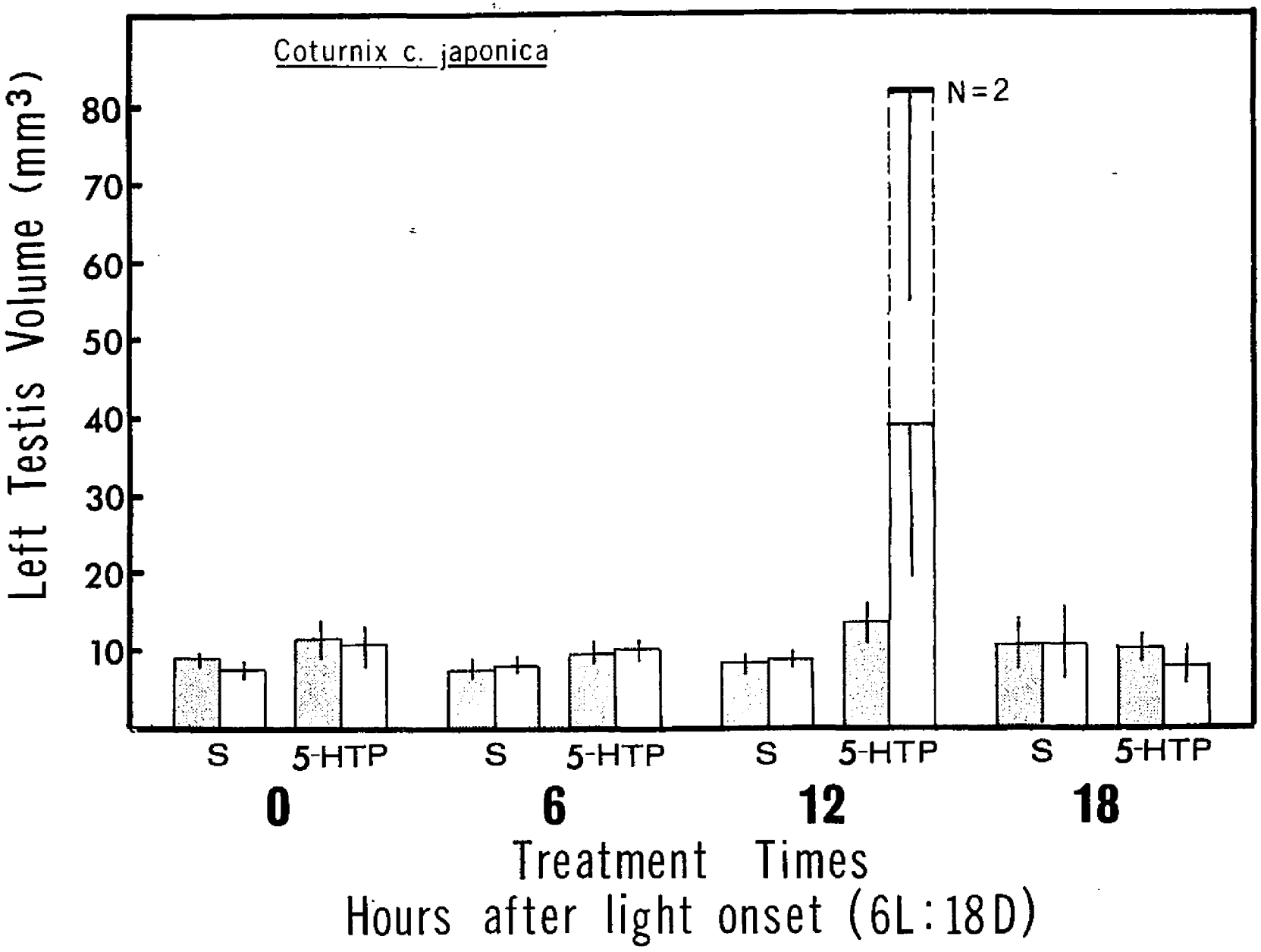

Coturnix c. japonica
N=2
Left Testis Volume (mm3)
80
70
60
50
40
30
20
10
S
5-HTP
S
5-HTP
S
5-HTP
S
5-HTP
0
6
12
18
Treatment Times
Hours after light onset (6L:18D)

Figure 4: Testis growth in house sparrows, Passer domesticus, induced by 5-hydroxytryptophan (5-HTP) and corticosterone injections made daily 12 hours before light onset (LD 8:16) for six weeks. Two additional groups received either L-dihydroxyphenylalanine (L-DOPA) or saline according to the same injection regimen. Testis condition was determined biweekly by laparotomy. 'Pre' denotes the mean testis volume of 10 birds prior to the start of injections.

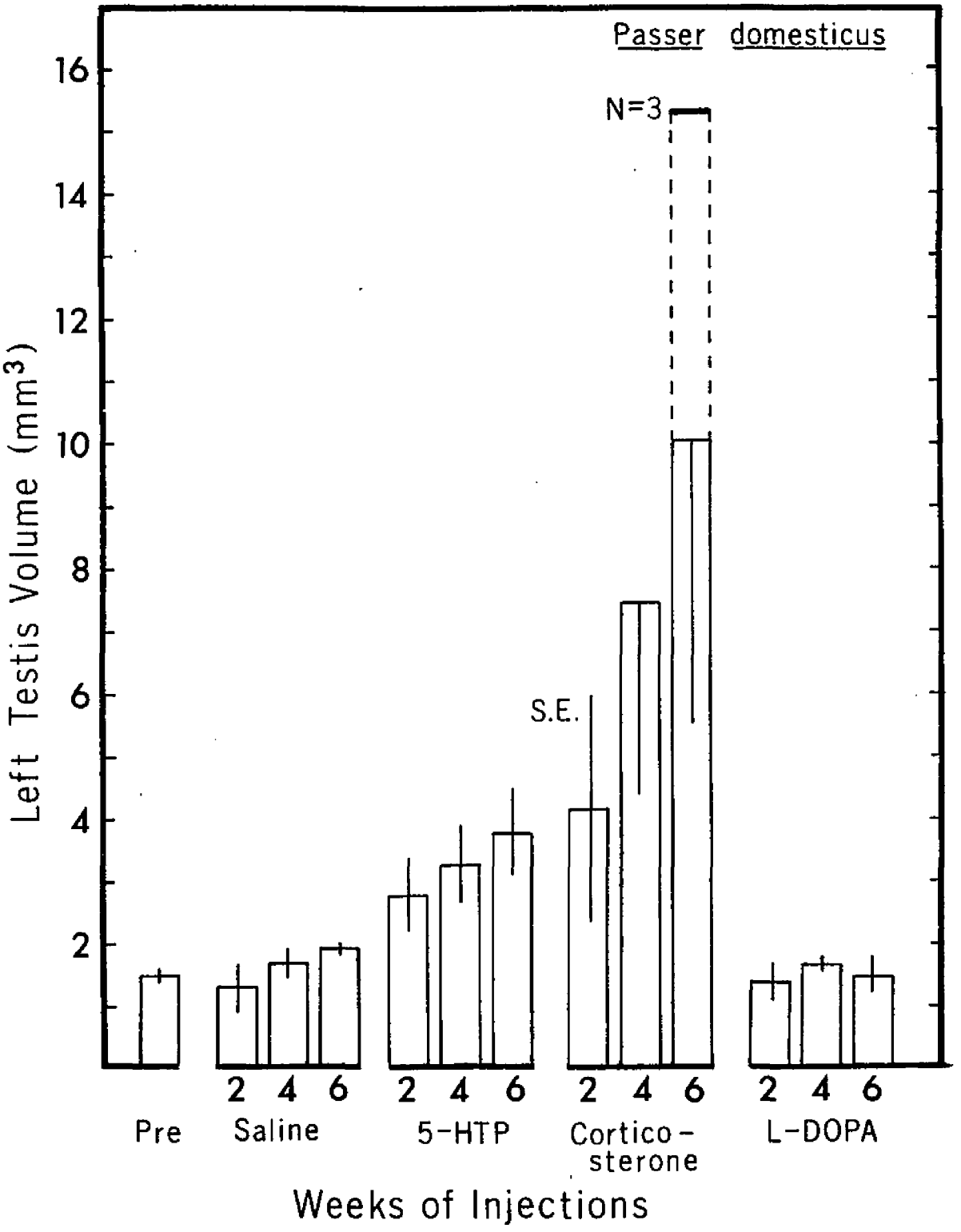
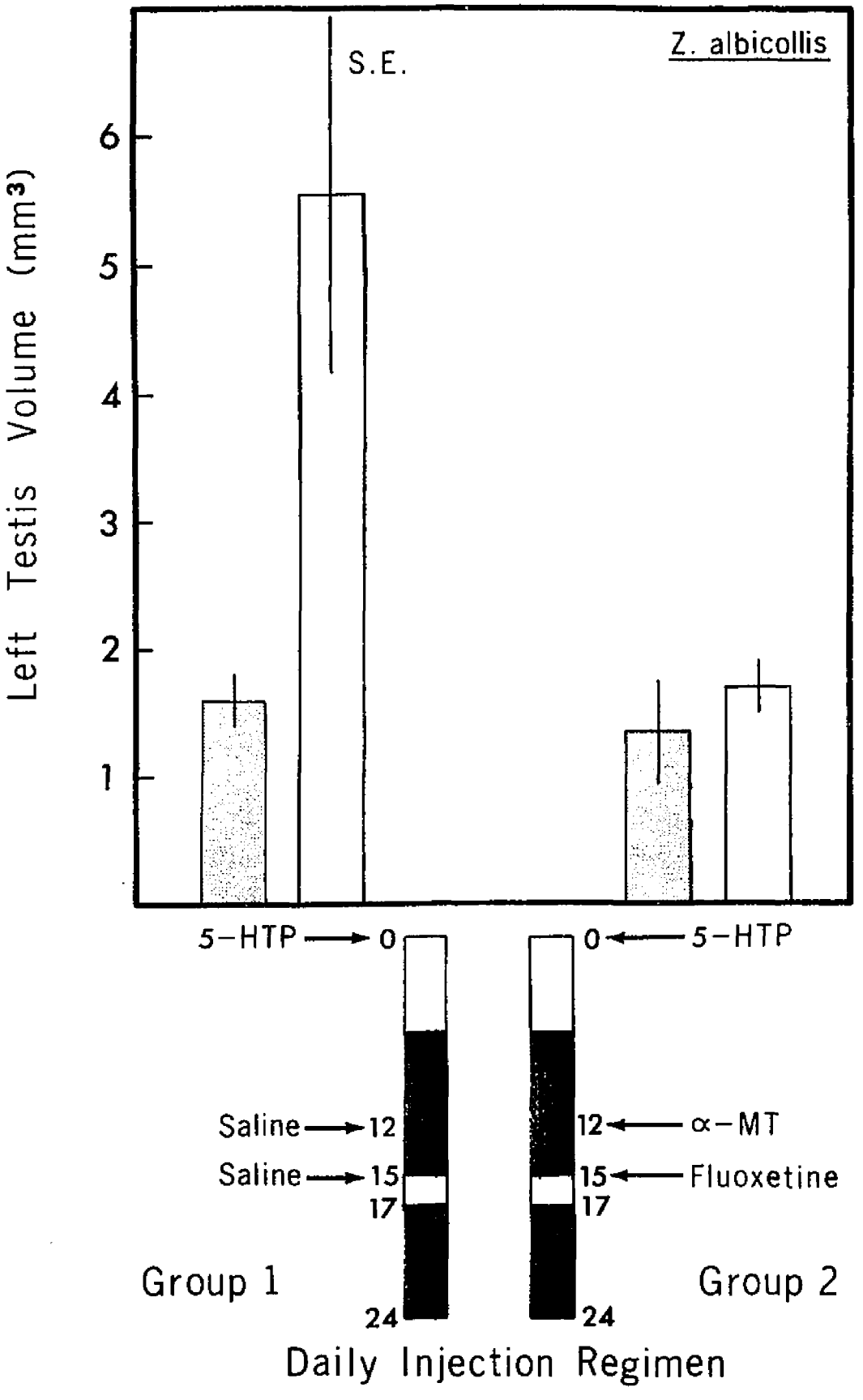

Passer domesticus
N=3
S.E.
Left Testis Volume (mm³)
16
14
12
10
8
6
4
2
2 4 6
Pre
Saline
5-HTP
Cortico-sterone
L-DOPA
Weeks of Injections

Figure 5. The effect of serotonergic-potentiating (5-HTP and fluoxetine) and catecholaminergic-inhibiting (α-MT) drugs on testis growth in white-throated sparrows maintained on an interrupted-night photoperiodic regime. Injections were made daily for 14 days. Birds were in winter, photosensitive condition. Testis volume was determined for each bird (6 birds per group) before (shaded bars) and after (open bars) treatment by unilateral laparotomy.


Z. albicollis
S.E.
Left Testis Volume (mm³)
6
5
4
3
2
1
5-HTP → 0
0 ← 5-HTP
Saline → 12
Saline → 15
17
12 ← α-MT
15 ← Fluoxetine
17
Group 1
Group 2
24
24
Daily Injection Regimen

DISCUSSION

The three experiments reported here suggest that serotonin-stimulating drugs can affect gonadal development in photosensitive birds when given at particular times of day. Injections of 5-HTP to quail on a short photoperiod (LD 6:18) stimulated gonadal growth when made 12 h before 'dawn' but not at 0, 6, or 18 hours beforehand (Experiment 1, fig. 3). Evidence from interrupted-night experiments indicates that the peak photoinducible period occurs about 13 h after onset of a short photoperiod in quail (Follett and Sharp, 1969); That is, coincidence of a brief light exposure at that time stimulated the highest rate of testis growth. In addition, a daily peak of plasma corticosterone occurs near dawn in quail on LD 6:18 (Boissin and Assenmacher, 1970). A daily rise in plasma corticosterone (CS) concentration is thought to entrain circadian rhythms of photosensitivity (Meier and Dusseau, 1973; see also review: Meier and Ferrell, 1978.) In quail then, the peak photoinducible phase may occur approximately 13 h after the daily rise of plasma corticosterone. Serotonergic activity which increases plasma CS levels (Section I) may also be capable of entraining photosensitivity rhythms. The peak photoinducible phase may also occur approximately 13 h after serotonergic stimulation with 5-HTP injections inasmuch as

injections of 5-HTP given only at 12 h before light onset stimulated testicular development in Experiment 1. If 5-HTP injections entrain a photosensitivity rhythm, then the peak photoinducible phase of that rhythm (at hour 13) would coincide with the onset of the 6-h light period only when 5-HTP injections were given 12 h beforehand, and not at the three other injection times. Therefore, the results support a hypothesis that serotonergic activity can entrain a rhythm of photosensitivity in quail, possibly by way of its positive influence on plasma corticosterone concentrations. The fact that 5-HTP injections 12 h before light onset did not stimulate gonadal development in all birds may indicate that the peak photoinducible phase occurs somewhat earlier or later than 12 h after the 5-HTP injections. Some birds may not have been influenced by 5-HTP injections.

In Experiment 2, gonadal growth occurred in house sparrows when injected with corticosterone 12 h before light onset (LD 9:15). Because corticosterone entrains several physiological rhythms including a photosensitivity rhythm in birds (see Introduction), the observed gonadal growth suggests that corticosterone injections entrained a rhythm of photosensitivity in house sparrows such that at least part of the short light period coincided with a photoinducible phase of that rhythm. Unfortunately, no data are available in house sparrows which relate the daily pattern of plasma CS levels with the photoperiod. However, using an interrupted-night

experiment, Menaker (1965) proposed that the peak photoinducible phase in house sparrows occurs approximately 12 h after dawn. Since a photoinducible period occurs approximately 12 h after both dawn and corticosterone injection, the daily rise of plasma CS levels may occur near dawn in house sparrows on short daylengths. Even so, the CS injections did not stimulate a full gonadal response. An alternate explanation, in which two photoinducible phases exist, could also account for the results of the present experiment with house sparrows, and those of studies utilizing interrupted-night experiments. Using reproductively stimulated house sparrows, Meier and Dusseau (1973) demonstrated that a disturbance (handling) entrained two photoinducible phases, one for luteinizing hormone (LH) and the other for follicle-stimulating hormone (FSH). Light is thought to be necessary during both photoinducible phases for full gonadal growth and maintenance to occur. Daily disturbances for six days (LD 16:8) reduced testes weights in groups handled one and 15 h before the onset of dark, but not at 8 or 20 h beforehand. The authors proposed that the photoinducible phase for FSH occurs 16-22 h after the disturbance while that for LH occurs approximately 4-8 h afterwards. If handling and injection (12 h before light onset) entrained those photoinducible phases in Experiment 3, then the photoinducible phase for LH would have occurred in the dark, whereas that for FSH would have occurred in the light.

Thus, some gonadal growth might be expected with FSH secretion alone. However, injections of mammalian FSH alone at different times of day and year failed to stimulate gonadal growth in house sparrows (MacGregor, M.S. thesis, L.S.U.). Secretion of both FSH and LH appears to be necessary to stimulate increases in gonadal weights. Thus while corticosterone injections entrained a photoinducible phase for FSH that coincided with the light period, the lack of LH secretion might explain the limited gonadal development with corticosterone injections.

To a lesser degree, 5-HTP injections given to house sparrows in Experiment 2 also caused gonadal growth whereas DOPA and saline injections had no effect. These results would further support a hypothesis that a plasma CS rhythm is mediated, at least in part, by serotonergic activity. Even so, not all 5-HTP treated birds responded to injections. The results may indicate, as with the corticosterone group, that only one of the photoinducible phases for FSH and LH secretion coincided with the light period. In addition, as suggested previously, (Section I), other systems (ie. cholinergic) may need to be stimulated to produce a full response. Likewise, the lack of response to DOPA injections does not necessarily preclude a role in photoentrainment. Additional injection times would clarify this question.

The results of Experiment 3 indicate that stimulation of serotonergic activity (fluoxetine) and inhibition of catecholaminergic activity (α-MT) during the photoinducible

period inhibited gonadal growth in white-throated sparrows on an interrupted-night photoperiodic regime. The response of a second group that received saline injections instead duplicates the results of a previous study (Jenner and Engels, 1952) in which testicular growth was observed in white-throated sparrows placed on an interrupted-night photoperiod.

Catecholaminergic neurons can be identified by fluorescence techniques. Fluorescence studies in reproductively stimulated Zonotrichia leucophrys gambelii, a close relative of white-throated sparrows, indicated that the photoinducible phase (hours 10-17 after light onset) coincided with the period (hours 10-15) of maximal fluorescence (high levels of catecholamine transmitters) in the palisade layer of the hypothalamus (Warren et al., 1973). In chickens, increased hypothalamic catecholamine levels occurred concomittant with increased gonadotropin activity (Graber and Nalbandov, 1972). Therefore, inhibition of catecholaminergic activity with α-MT in Experiment 3 might account for the observed inhibition of gonadal growth. Potentiation of serotonergic activity may also account for the inhibition of gonadal growth. In Japanese quail, 5-HTP inhibited photoperiodically-induced testicular growth (El Halawani et al., 1978) whereas injections of 5, 6 dihydroxytryptamine (serotonin neurotoxin) augmented testicular development (Calas, 1975).

Based on studies with white-throated sparrows, Meier and Dusseau (1973) proposed that a model that includes two photoinducible phases is involved in photostimulation of the reproductive system and can also account for the results of studies utilizing interrupted-night experiments. Essentially, an entraining interval of light is thought to set both the photoinducible phases for LH and FSH release. The former occurs during the entraining light interval while the latter occurs later in the day. Light interruption that coincides with any part of the photoinducible phase for FSH causes the release of FSH and stimulates gonadal growth. The inhibition of catecholaminergic activity (α-MT) and/or the stimulation of serotonergic activity (fluoxetine) near the anticipated photoinducible phase for FSH may have inhibited those pathways involved in FSH release and thereby suppressed gonadal growth.

Experiments 1 and 2 in this section have demonstrated that 5-HTP injections can entrain rhythms of photsensitivity in two other avian species. Since both injection groups in Experiment 3 received 5-HTP, additional groups would be needed to discriminate whether it can influence entrainment of the photosensitivity rhythm during an interrupted-night photoperiodic regime. Further studies are needed to elucidate serotonergic entrainment of photosensitivity rhythms and to determine whether the timing of drugs (α-MT and fluoxetine, Experiment 3) near the second light period is indeed critical for inhibition of gonadal growth.

SECTION III

TEMPORAL SYNERGISM OF 5-HYDROXYTRYPTOPHAN (5-HTP) AND DIHYDROXYPHENYLALANINE (DOPA) WHICH INFLUENCE SEASONAL CONDITIONS IN WHITE-THROATED SPARROWS AND HOUSE SPARROWS

INTRODUCTION

During the last several years prolactin has been shown to have a variety of activities in birds. Many of its activities vary depending on the time of daily injection or on the temporal relation with the daily rhythm of corticosterone. Daily injections of prolactin given to white-throated sparrows produced variable effects on fat stores (Meier and Davis, 1967) and occurrence of nocturnal activity (Meier, 1969b) depending on the time of day (LD 16:8) when injections were made. Daily rhythms of corticosterone apparently entrain circadian rhythms of responsiveness to prolactin. Under constant light, which avoids photoperiodic entrainment, fat stores (Meier and Martin, 1971), gonadal growth (Meier et al., 1971b) and oriented nocturnal activity (Martin and Meier, 1973) were stimulated or inhibited depending on the times of prolactin injections relative to daily injections of corticosterone.

Mammalian regulation of prolactin release appears to be by way of a hypothalamic prolactin inhibiting factor (PIF). That is, high chronic levels of PIF inhibit prolactin release. While there is evidence for a PIF in ducks (Tixier-Vidal and Gourdji, 1972), studies of other avian species suggest that prolactin regulation occurs via a stimulatory factor (PRF) (reviews: Kobayashi and Wada, 1973; Meites, 1977). Furthermore, separate mechanisms for production and release may exist. Pituitary prolactin con-

tent varies during the day in the white-throated sparrow (Meier et al., 1969) and duck (Ensor, 1975). A daily rise was observed during the morning in May as well as August in the sparrow. The time of daily release (rise in plasma content) of prolactin, however, varies seasonally: during the afternoon in May and late during the night in August. Thus avian prolactin regulation and the identitites of stimulatory or inhibitory hypothalamic substances is still in question.

The control of prolactin release in mammals is thought to be by way of dopaminergic activity. Injections of DOPA (dopamine precursor) caused a rapid (30 minutes), dose-related decrease in plasma prolactin levels in rats (Jimenez et al., 1978; Langer et al., 1978). Dopamine is carried from hypothalamic neuron terminals in the median eminence to the adenohypophysis by portal vessels (Ben-Jonathan et al., 1977; Plotsky et al., 1978). Dopamine is probably mammalian PIF (Shaar and Clemens, 1974; Horowski and Graf, 1976; Gibbs and Neill, 1978).

Daily rhythms of plasma prolactin and corticosterone are believed to be the hormonal expressions of two circadian neural oscillators. These daily rhythms change in phase with one another throughout the year in white-throated sparrows (Meier and MacGregor, 1972). This changing phase relationship is thought to regulate the occurrence of seasonal reproductive and migratory conditions. At one

season the daily rise of plasma prolactin coincides with a tissue-sensitive phase entrained earlier in the day by the corticosteroid rhythm. This phase relationship may stimulate fattening and reproductive development whereas at another season the time of daily prolactin release does not coincide with a sensitive phase and thus results in loss of fat stores and gonadal regression. Prolactin injections given to white-throated sparrows at different times during the day apparently imposed a new phase relationship with the daily corticosterone rhythm. Therefore, some prolactin injection times coincided with a sensitive phase whereas others did not and may account for the variable effects observed in fat stores, gonadal growth, and nocturnal activity after such treatment.

Prolactin injections then, appear to reorganize one central neural oscillator into a new daily rhythm. Dopaminergic activity appears to regulate the endogenous daily prolactin rhythm by feedback mechanisms and thus may also be thought of as an expression of that circadian oscillation. Therefore, drug injections which stimulate dopaminergic activity may mimic prolactin injections and reset the oscillation. Subsequent induction of similar variable effects may occur in white-throated sparrows depending on the time of daily injection.

Serotonergic activity, as stimulated by 5-HTP injections, raised plasma corticosterone concentrations in quail and white-throated sparrows (Section I) and appears to be im-

portant in maintaining the daily rhythm of plasma corticosterone in quail (Boissin and Assenmacher, 1971). Serotonergic and catecholaminergic activity then, appear to be important in the regulation of corticosteroid and prolactin secretion. These hormones, in turn, form the basis of a temporal synergism controlling gonadal growth, fattening and migratory behavior. These conditions may similarly be induced by way of stimulating serotonergic and catecholaminergic activity by drugs. Therefore, in the following experiments sparrows were given daily injections of DOPA (catecholaminergic) at one of several hourly intervals after daily injections of a serotonergic stimulant (5-HTP).

MATERIALS AND METHODS

Experiment 1. Male white-throated sparrows were removed from an outdoor aviary in March, examined for reproductive and fat conditions, and housed indoors individually in small cages (18 x 26 x 26 cm). The reproductive condition of each bird was determined by unilateral laparotomy as described previously (Section II). Subcutaneous fat stores in the ventral abdominal and furcular regions were estimated using an index described by Weise (1956). After several days, on March 19, daily injections of DOPA and PCPA (serotonin synthesis inhibitor) were initiated in four groups at 3, 8, 12, or 18 hours after injections of 5-HTP at light onset (LD 9:15). Injections of prolactin 4, 8 or 12 h after injections of corticosterone have been shown to stimulate conditions appropriate for fall, summer, or spring, respectively, in white-throated sparrows (see review, Meier and Ferrell, 1978). A fifth group received two daily injections of 0.9% saline 3 h apart. A sixth group received a single daily injection of PCPA. On March 21, all birds were placed in continuous light for the duration of the injection period. Long days or continuous light in March stimulate the onset of spring reproductive and migratory activity. Drug injections were begun while birds were still on a shortened daylength in order to establish a new phase relationship between the two circadian oscillators before the birds were transferred to continuous light. On April 1, injections

were terminated, gonadal development and fat stores were ascertained, and all birds were transferred to long daily photoperiods (LD 15:9). Additional examinations were made at 2, 4, and 6 weeks post-injection.

Experiment 2. This experiment was performed to study the catecholaminergic activities, dopaminergic and noradrenergic, that may be important in a synergism with timed serotonergic activity (Experiment 1). A 4-h injection interval between corticosterone and prolactin has been shown to inhibit gonadal development in white-throated sparrows (Meier et al., 1971b). Therefore, differential gonadal inhibition may occur depending upon which catecholaminergic activity is high 3 to 4 hours after serotonergic stimulation. Inasmuch as injections of DOPA alone, as done in Experiment 1, increase levels of both dopamine and noradrenaline, additional drugs were employed to potentiate selectively, either amine.

The drug DDC (Appendix 1) inhibits the enzyme that converts dopamine to noradrenaline. Therefore, a simultaneous injection of DOPA and DDC should stimulate dopaminergic activity but inhibit noradrenergic activity. The drug α-MT (Appendix 1) inhibits the conversion of the amino acid tyrosine to DOPA while the drug DOPS is a specific precursor for noradrenaline. Consequently a simultaneous injection of α-MT and DOPS should selectively inhibit dopaminergic activity. In mice, this combination of drugs caused a 50%

depletion of dopamine in the central nervous system without change in noradrenaline levels (Creveling et al., 1967). All of these drugs are capable of entering the central nervous system of mammals via the peripheral circulation (see Appendix 1).

Female white-throated sparrows were removed from an outdoor aviary on March 23 and housed indoors individually in small cages. Four birds were killed at this time to determine pre-experimental ovary and oviduct weights. On March 24, daily injections were initiated in two groups of four birds each as follows: 1) 5-HTP and PCPA followed in 3 h by DOPA and DDC (150 mg/kg) and 2) 5-HTP and PCPA followed in 3 h by α-MT (400 mg/kg) and DOPS (150 mg/kg). On March 26, the photoperiod was changed from LD 9:15 to continuous light until the last day of injections on April 5. Thus the birds had 13 days of injections and 11 days of continuous light. A third group of birds received the same photoperiodic treatment but received no injections. On April 6, all birds were killed and ovary and oviduct weights taken.

Experiment 3. Because an 8-h interval between corticosterone and prolactin injections induces late summer conditions in white-throated sparrows, including inhibition of gonadal development (Meier et al., 1971b), an 8-h interval of drug injections was employed to test whether or not gonadal development could be altered by temporal manipulation of serotonergic and catecholaminergic activity.

Female white-throated sparrows were removed from an outdoor aviary on June 7 and housed indoors, individually, in small cages. Seven birds were killed at this time to determine ovary and oviduct weights prior to the experiment. From June 9-22, birds were maintained on continuous light and divided into three injection groups as follows: 1) 5-HTP followed in 8 h by DOPA (5-HTP: 8, DOPA); 2) corticosterone followed in 8 h by DOPA (CS: 8, DOPA); and 3) 5-HTP followed in 8 h by 0.9% saline (5-HTP: 8, saline). A fourth group of birds was exposed to continuous light only. Each group contained five birds. On June 23, all birds were killed to determine ovary and oviduct weights.

Experiment 4. A similar experiment was performed with female house sparrows, Passer domesticus, concurrently with Experiment 3. Daily prolactin injections 8 h after corticosterone injections stimulate gonadal development in this species whereas a 16-h injection interval is inhibitory (Meier et al., 1971b). Accordingly, drug injections were made at these hourly intervals to test for a temporal synergism of neural activity controlling testicular growth.

Female house sparrows were captured during May and early June from a resident population near the Louisiana State University campus. They were housed approximately three weeks prior to the experiment in an outdoor aviary. Seven birds were killed and examined prior to the start of the injections. From June 9-22 two groups of birds kept in con-

tinuous light received daily injections of 5-HTP followed in either 8 hours (5 birds) or 16 hours (6 birds) by DOPA injections. Ovary and oviduct weights were determined on June 23.

RESULTS

Experiment 1. The testis volumes of white-throated sparrows in all groups averaged less than one mm^3 prior to the experiment. The mean post-injection testis volume (11 mm^3) of birds that received saline injections indicates that the sparrows were photosensitive and photostimulated in continuous light.

Daily injection of DOPA and PCPA at varying time intervals after 5-HTP injections produced variable responses in testicular growth and fat stores. According to a one-way analysis of variance, the differences in testis volume resulting from variations in treatment times are significant ($p \leq .05$) at 0, 2, (fig. 6) and 6 weeks post-injection period. Following injections (0 weeks), the largest testes were found in the group that received 5-HTP: 12, DOPA-PCPA injections (mean $\pm$ S.E.: 21 $\pm$ 7.5 mm^3). Smaller testes were found in the 3-and 8-hour groups (5 $\pm$ 0.4 mm^3, respectively) and in the 18-h group (7 $\pm$ 3.1 mm^3). The mean testis volumes in the 3- and 8-h groups were significantly smaller than that of the saline-injected group (11 $\pm$ 2.6 mm^3) ($p \leq .05$, Student's *t*-test). Testes weights of saline-injected birds did not differ significantly from those of birds injected with PCPA only (12.6 $\pm$ 2.4 mm^3). Biweekly laparotomies after the injection period revealed that the injections did not permanently repress testis growth in any

bird. Testis growth peaked at approximately four week post-injection when testis volumes were similar in all groups. However, the injections apparently influenced the rates of growth as evidenced by differences in testis volumes at 2 (fig. 6, upper) and 6 weeks post-injection ($p < .05$, analysis of variance). At 2 weeks, the mean testis volume in the 3-h (23.8 ± 5.5 mm^3), 8-h (31.3 ± 4.6 mm^3) and 18-h (31.7 ± 15.8 mm^3) groups remained significantly less ($p < .05$, t-test) than that in the 12-h group (72.5 ± 14.5 mm^3). At six weeks, testes had regressed in the 3-h group (10.9 ± 6.7 mm^3) and were significantly less ($P < .01$) than that of the 12-h group (82.3 ± 16.8 mm^3).

Fat stores also varied with the time interval of drug treatment. These differences did not become apparent until two weeks after the end of injections (fig. 6, upper). The highest fat stores occurred in the 12-h group which also had the largest testes. High fat stores were also seen in the 3-h group which had relatively small testes. Fat levels were lower in the 8-h group than those of either the 3- or 12-h groups ($p < .05$, Student's t-test). The differences were even more pronounced at 4 weeks post-injection ($P \leq .02$). According to an analysis of variance, the variation in fat stores at four weeks is significant ($p = .02$) among the four time groups.

Thus, in general, the 12-h treatment was characterized by large gonadal size and high fat stores. In contrast, the

8-h treatment inhibited gonadal growth and promoted low fat levels. The 3-h treatment inhibited gonadal growth and may have elevated fat stores somewhat.

Experiment 2. Injections designed to selectively inhibit noradrenaline levels (DOPA and DDC) 3 h after 5-HTP injections suppressed ($p < .05$, Student's t-test) the normal photoperiodic increase in oviduct weights noted in untreated white-throated sparrows (16.9 ± 0.3 mg vs 22.8 ± 2.7 mg, respectively) (fig. 7). However, selective inhibition of dopamine (α-MT and DOPS) 3 h after 5-HTP injection raised oviduct weights slightly (28.3 ± 4.5 mg), though not significantly compared with those of untreated birds. The oviduct weights of the group in which noradrenaline levels were inhibited (16.9 ± 0.3 mg) were less ($p = .05$, t-test) than those of the group in which dopamine levels were inhibited (28.3 ± 4.5).

Experiment 3. Oviduct weights of female white-throated sparrows increased somewhat in all treatment regimens compared with pre-experimental levels (fig. 8). However, oviduct weights of both the 5-HTP; 8, DOPA group (23.6 ± 1.6 mg) and the CS: 8, DOPA group (25.8 ± 3.3 mg) were significantly less ($p < .01$, Student's t-test) than those of either the 5-HTP: 8, saline (38.2 ± 3.2 mg) or light-only groups (35.6 ± 5.0 mg). Ovary weights in all treatment groups did not vary significantly from pre-experimental levels.

Experiment 4. Female house sparrows treated in a manner similar to the female white-throated sparrows reported above also exhibited a differential oviduct response which varied in relation to the time at which DOPA injections were given after 5-HTP. 5-HTP: 16, DOPA injections suppressed ($p < .01$, Student's t-test) oviduct weights (17.0 $\pm$ 1.8 mg) compared with pre-experimental levels (32.7 $\pm$ 4.3 mg) whereas 5-HTP: 8, DOPA injections did not significantly alter oviduct weights (34.9 $\pm$ 6.5 mg) (fig. 9). Oviduct weights in the 8-h group were significantly higher ($p = .01$) than those in the 16-h group. Ovary weights did not differ among any groups.

Figure 6. The temporal synergism of 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA) in controlling testis development and fattening in photosensitive white-throated sparrows. Daily injections of DOPA and para-chlorophenylalanine (PCPA) were given 3, 8, 12, or 18 h after daily injections of 5-HTP. A fifth group received daily injections of PCPA. Injections were given for 14 days while birds were maintained in continuous light. Thereafter, the photoperiod was LD 16:8. Measurements were made immediately after injection period (lower) and two weeks later (upper). The dashed and dotted lines are the mean testis volumes (closed circles) and fat indices (open circles), respectively, of birds injected with saline twice daily in a 3-h relation. Testis volumes were calculated (ellipsoid formula) after laparotomy operation and subcutaneous fat stores were estimated by an index after Weise (1956). A value of zero indicates no fat in furcular or abdominal areas; 40 represents the maximum possible.

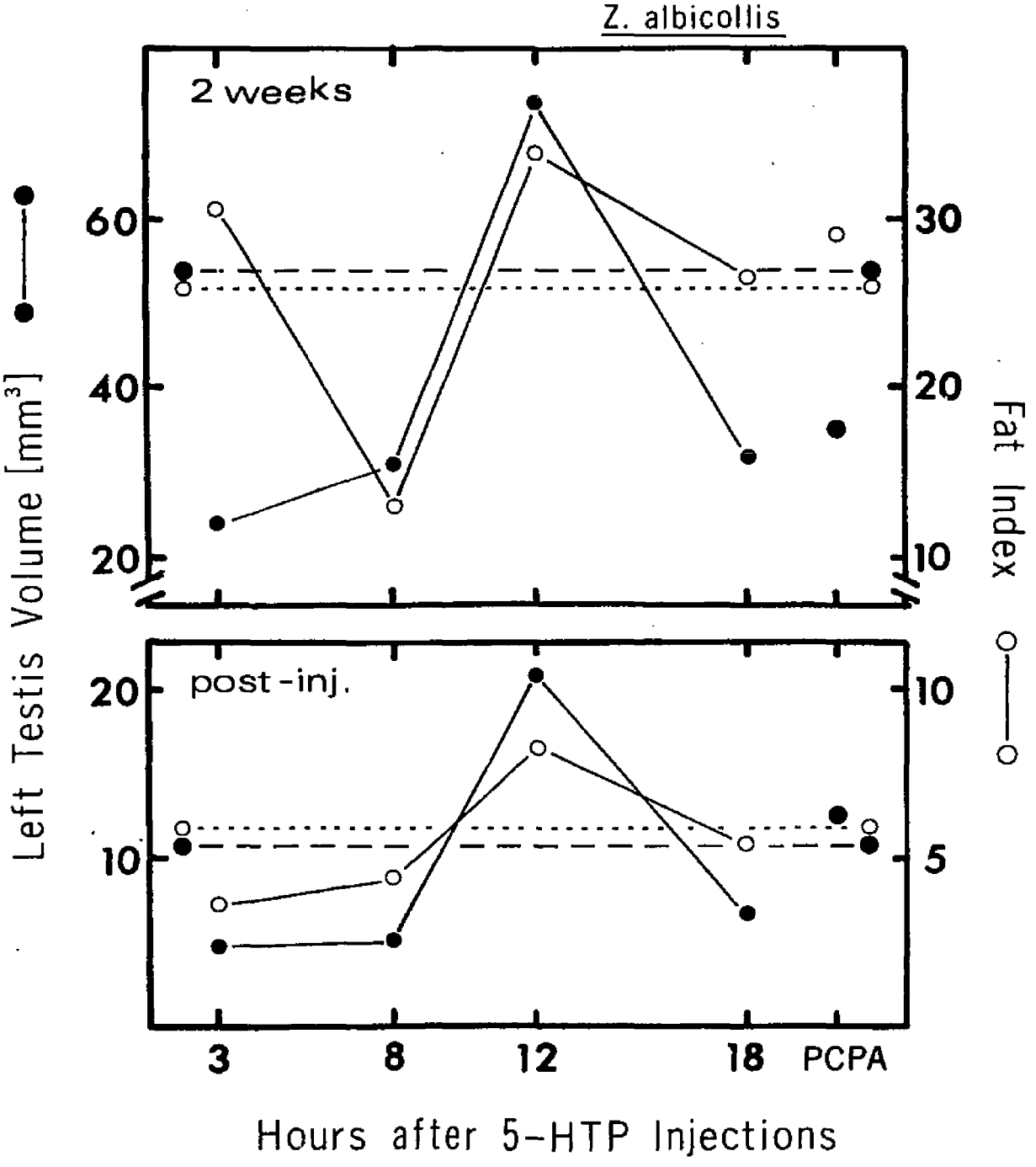

Z. albicollis
2 weeks
post-inj.
Left Testis Volume [mm³]
Fat Index
60
40
20
30
20
10
10
5
3
8
12
18
PCPA
Hours after 5-HTP Injections

Figure 7. Oviduct development in photosensitive white-throated sparrows when either dopamine or noradrenaline levels are specifically stimulated by drug treatment three hours after simultaneous 5-HTP and PCPA injections. DOPA and DDC combination potentiates dopaminergic activity; α-MT and DOPS enhances noradrenergic activity. Four birds were exposed to continuous bright light (LL) only. Injections were daily for 14 days. See Appendix 1 for further explanation of drugs.

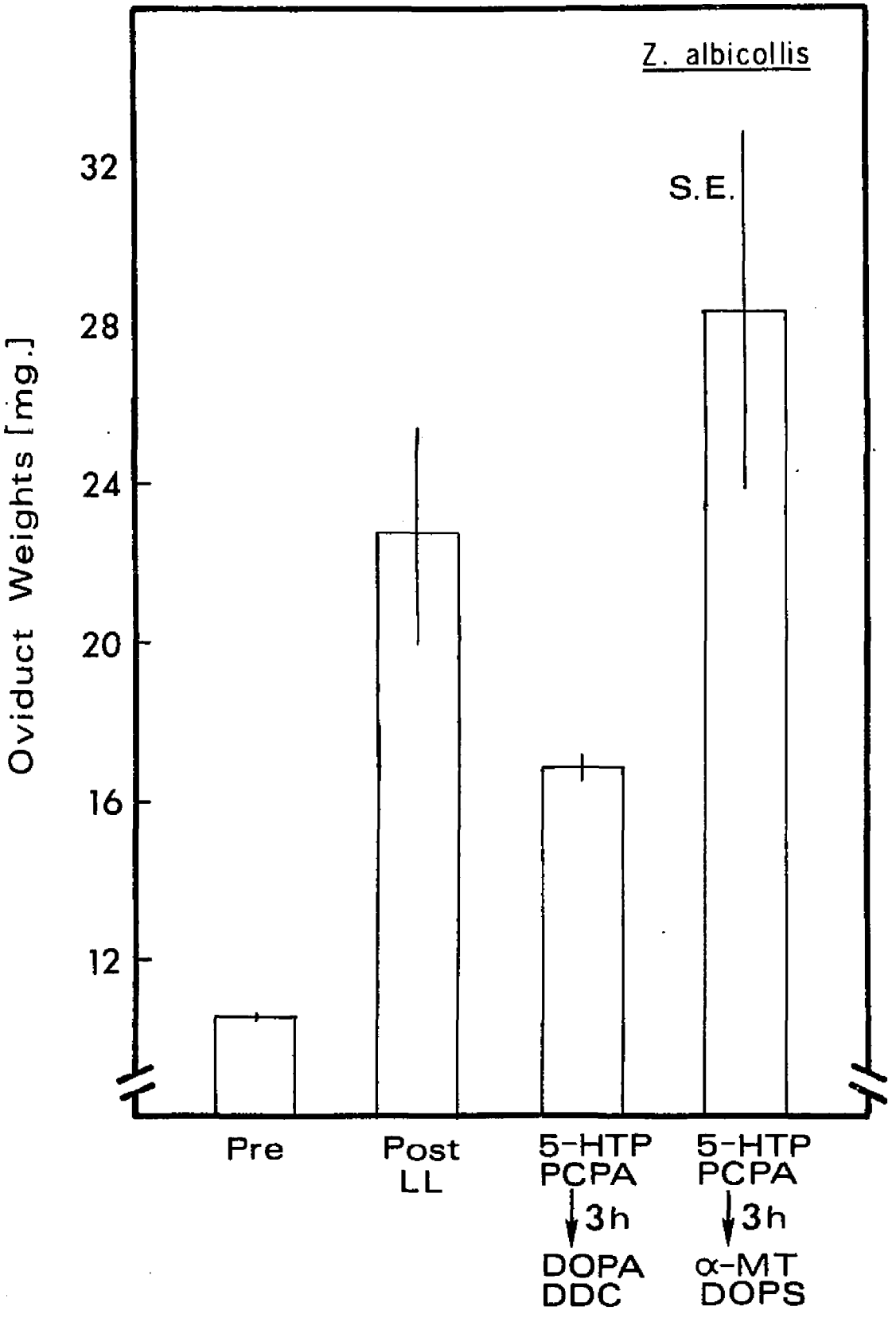

Z. albicollis
S.E.
Oviduct Weights [mg.]
32
28
24
20
16
12
Pre
Post LL
5-HTP PCPA
3h
DOPA DDC
5-HTP PCPA
3h
α-MT DOPS

Figure 8. Oviduct weights of white-throated sparrows following daily injections of DOPA 8 hours after daily injections of either 5-HTP or corticosterone. Injections were given for 14 days while birds were in continuous light.

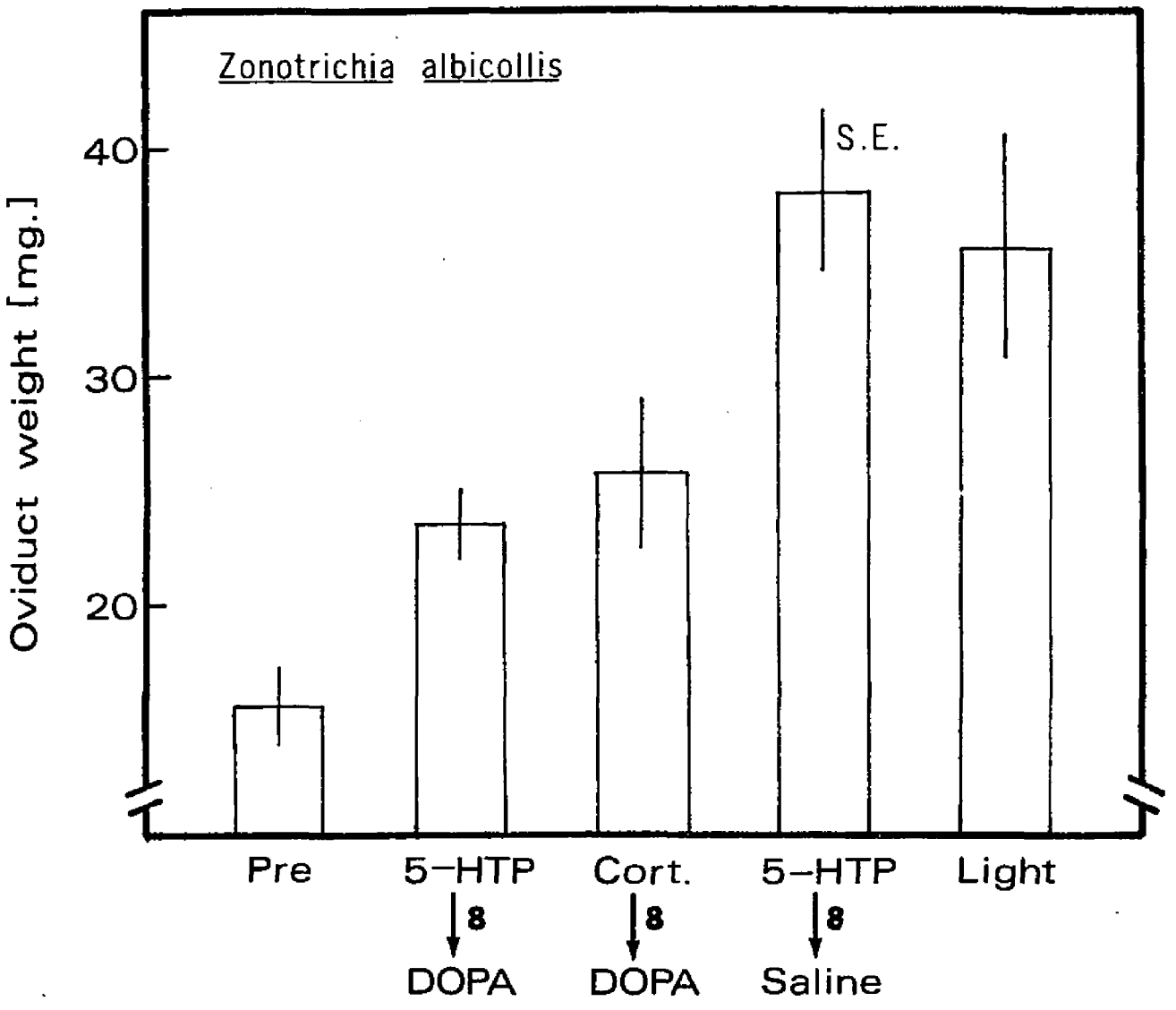
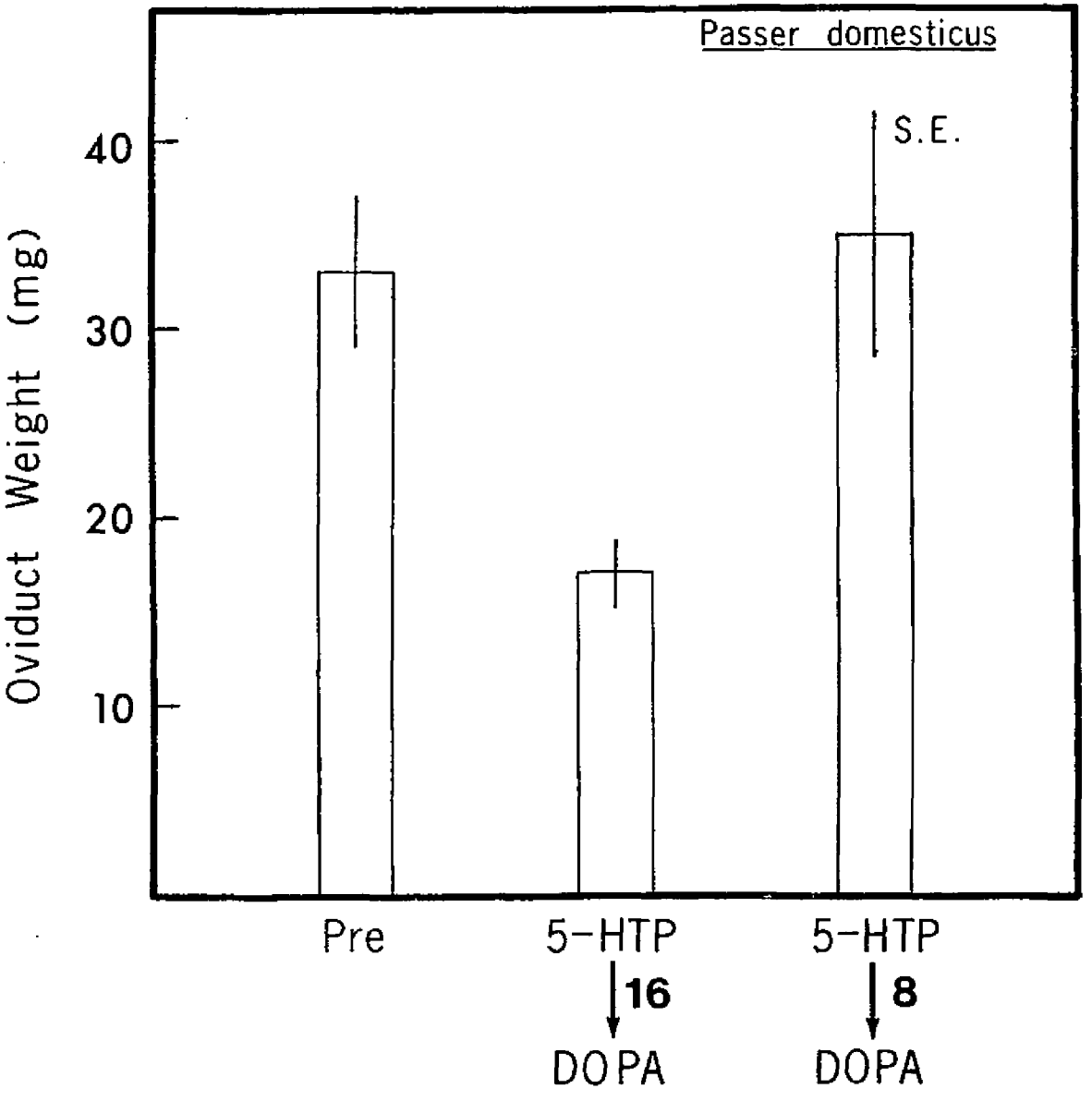

Zonotrichia albicollis
Oviduct weight [mg.]
40
30
20
S.E.
Pre
5–HTP
8
DOPA
Cort.
8
DOPA
5–HTP
8
Saline
Light

Figure 9. Oviduct weights of house sparrows following daily injections of DOPA at 8 or 16 hours after daily 5-HTP injections. Injections were given for 14 days while birds were in continuous light.


Passer domesticus
S.E.
Oviduct Weight (mg)
40
30
20
10
Pre
5-HTP
16
DOPA
5-HTP
8
DOPA

DISCUSSION

The experiments in this section were designed to elucidate the reproductive and fattening effects of timed injections of drugs which alter serotonergic and catecholaminergic activities. In general, the temporal patterns of 5-HTP - DOPA injections which cause differential responses in gonadal growth and fattening (Experiment 1) are the same patterns as those of corticosterone - prolactin injections that induce similar responses in white-throated sparrows (Meier et al., 1971b; Meier and Martin, 1971). In Experiment 1, the 5-HTP: 12, DOPA-PCPA injections stimulated gonadal development and fattening. Daily injections of prolactin 12 h after corticosterone injections also stimulated reproductive and fattening responses. These conditions are similar to those found in white-throated sparrows during late spring. An 8-h drug regimen (5-HTP: 8, DOPA-PCPA) inhibited gonadal development and fattening. An 8-h corticosterone - prolactin injection interval produced similar results and approximate the conditions that occur naturally during late summer. Finally, a 3-h drug regimen (5-HTP: 3, DOPA-PCPA) inhibited gonadal development and may have stimulated fattening, results that are similar to those reported for a 4-h corticosterone-prolactin injection regimen. The conditions observed after either injection regime are similar to those seen in white-throated sparrows during the fall migratory period.

Injection of DOPA (Experiment 1) was expected to stimulate both dopamine and noradrenaline synthesis. Since a 3-h drug regimen inhibited gonadal development, Experiment 2 was designed to distinguish the catecholamine which may be more important for gonadal inhibition at that time interval. Selective potentiation of dopaminergic activity by DOPA and DDC injections also suppressed reproductive (oviduct) development whereas specific inhibition of dopaminergic activity by α-MT and DOPS injection did not significantly affect oviduct weights. The results indicate that stimulation of dopaminergic activity 3 h after serotonergic stimulation inhibits gonadal growth in white-throated sparrows. The 8-h interval of drug treatment (5-HTP: 8, DOPA) resulted in inhibition of reproductive development in both male (Experiment 1) and female (Experiment 3) white-throated sparrows.

The results of experiments with white-throated sparrows in this section prompt the following conclusions. First, daily injections of 5-HTP can entrain a rhythm of sensitivity to DOPA injections; That is, DOPA produced variable effects on gonadal development and fat stores depending on the time of its daily injection after 5-HTP. The conditions observed from timed drug injections are similar to those that result from daily injections of corticosterone and prolactin given in the same hourly relations. Treatment with specific catecholaminergic-affecting drugs (Experiment 2) suggests

that the temporal synergism influencing gonadal growth and fat stores involves serotonergic and dopaminergic activities. Inasmuch as both activities have been implicated in the regulation of plasma levels of corticosterone (see Section I) and prolactin (Introductin, this section), the results from both drug and hormone - injection experiments may involve the same temporal mechanisms.

Oviduct growth in house sparrows also responds differentially to the temporal administration of 5-HTP and DOPA. A 16-h interval depressed oviduct weights compared with the 8-h group which maintained oviducts near pre-experimental levels. Meier et al. (1971b) found that a temporal synergism of corticosterone and prolactin controlled testicular growth in house sparrows. Heaviest testes were found when prolactin was injected 8 h after corticosterone and lighest testes resulted after a 16-h interval. Although selective catecholaminergic injections were not given to house sparrows, the results suggest that a temporal synergism of serotonergic and catecholaminergic (possibly dopaminergic) activity influences gonadal growth, as proposed for white-throated sparrows.

Numerous studies (see Introduction) of neurotransmitter regulation of the reproductive system have failed to consider the possibility of circadian involvement. Serotonin has been regarded as inhibitory and either dopamine or noradrenaline as stimulatory for gonadotropin release. Since both

serotonergic and catecholaminergic drugs were given to each bird in the present experiments, one might have predicted on the basis of previous work that stimulation of both systems would have cancelled each other.

Circadian rhythms must be considered in any model of neurotransmitter involvement in the regulation of gonadal growth and fat stores in white-throated sparrows and gonadal growth in house sparrows. A temporal synergism between corticosterone and prolactin was proposed to account for the control of these conditions (Meier et al., 1971b). My results indicate that timed neurotransmitter activity should be included as elements of this model. A rhythm of plasma corticosterone is thought to be maintained by serotonergic activity. Stimulation of either by injections apparently reorganizes the same circadian neural oscillator which, in turn, entrains daily rhythms of tissue sensitivity. In the second part of the model, a daily rhythm of prolactin is regulated by dopaminergic activity. The daily rise of plasma prolactin stimulates dopaminergic activity by way of a negative feedback system. Both activities are considered to be the metabolic expressions of a second circadian neural oscillator. Stimulation of either by daily injections either stimulates or inhibits gonadal growth and fattening depending on the time of injections relative to daily injections of either corticosterone or 5-HTP. The coincidence of a tissue-sensitive phase with the daily rise in prolactin

levels and dopaminergic activity is thought to stimulate gonadal growth and fattening. The model accounts for the similarity between results obtained in both white-throated sparrows and house sparrows by timed drug injections and those obtained by Meier and colleagues with timed hormone injections.

In white-throated sparrows, daily injections of prolactin 12 h after corticosterone stimulated gonadal growth (Meier et al., 1971b), increases in fat stores (Meier and Martin, 1971), and northward-oriented nocturnal activity (Martin and Meier, 1973). These conditions are found in white-throated sparrows during the spring migratory period. Recently, a two week period of daily prolactin injections 12 h after corticosterone apparently reset the annual cycle of photorefractory white-throated sparrows as determined by several months of subsequent observation (Ferrell, 1979; Meier et al., in press). Initially, spring conditions were induced similar to those obtained previously with 12-h hormone injection regimens. Spring conditions were subsequently replaced by summer and fall conditions in a manner similar to the natural seasonal progression. Clearly, timed injections of corticosterone and prolactin that reset annual cycles must influence basic neural regulatory centers which control seasonal conditions. I believe that the results from this section with timed injections of monoamine-affecting drugs suggest the means by which hormone

injections affect those neural centers. The model of a monamine-hormone temporal synergism that controls gonadal growth and fat stores in white-throated sparrows may be appropriate for further study of the processes which regulate the annual cycle. Experiments reported in the following section tested the possibility that timed neurotransmitter activity through drug injections may influence the annual cycle in white-throated sparrows.

SECTION IV

RESETTING THE ANNUAL CYCLE IN WHITE-THROATED SPARROWS WITH SEROTONERGIC AND CATECHOLAMINERGIC DRUGS

INTRODUCTION

Although external cues such as daylength may be synchronizers of avian annual cyles, an increasing number of studies indicate that seasonal occurrences of gonadal development, fattening, molt and migratory activity are controlled by endogenous circannual rhythms which persist in constant conditions for several years (review: Gwinner, 1977). Furthermore, not all functions undergo circannual rhythms in some species. In white-crowned sparrows (Zonotrichia leucophrys gambelii) maintained on LD 20:4, molt and fattening recurred periodically (King, 1968) whereas testicular growth did not (Farner and Lewis, 1971; Sansum and King, 1976). A similar pattern occurred in white-throated sparrows (Z. albicollis) maintained on constant short (LD 8:16) (Weise, 1962) or long daily photoperiods (LD 16:8) (Meier and Fivizanni, 1975). In addition, when white-throated sparrows were kept for a year on LD 16:8 beginning in late spring, two periods of nocturnal activity occurred at approximately the seasons in which migratory activity occurs naturally (personal observation).

The regulation of endogenous annual cycles may involve seasonal changes in the phase relationship between two circadian hormone rhythms. The daily rhythms of plasma corticosterone in white-throated sparrows are different in May and August although daylengths are similar at those

seasons (Dusseau and Meier, 1971; Meier and Fivizanni, 1975). Similarly, the release of prolactin occurs in the afternoon in May but late at night in August (Meier et al., 1969). In late spring the daily rise in plasma prolactin levels occurs approximately 12 h after the daily rise in plasma corticosterone concentrations. From late summer to autumn the hormonal interval shifts from approximately 6-8 h, to 4 h, respectively. A period of daily injections of corticosterone and prolactin given in a 12-h relation reset the annual cycle of photorefractory white-throated sparrows into a late spring position (Ferrell, 1979; Meier et al., in press). On constant 14-h daily photoperiods following treatment, spring conditions were subsequently replaced by summer and fall conditions in a manner approximating the natural seasonal progression.

A change in phase of the circannual cycle cannot be explained by effects on peripheral systems but must involve the reorganization of central neural centers which direct metabolic and behavioral conditions by way of the neuroendocrine system. By using timed drug injections, stimulation of catecholaminergic activity 12 h after stimulation of serotonergic activity induced spring conditions in white-throated sparrows (Section III). A model was presented in Section III which accounts for the effects of timed hormone and drug injections. Inasmuch as hormone injections reset the annual cycle in white-throated sparrows, the model

predicts that timed drug injections may also influence the annual cycle. Thus, the following experiments tested the possibility that timed injections of serotonergic and catecholaminergic-affecting drugs may reset the annual cycle of white-throated sparrows.

MATERIALS AND METHODS

1977 Experiments

The first set of experiments with white-throated sparrows in autumn condition was started in October, 1977. Birds were taken from an outdoor aviary and housed individually in small (18 x 26 x 26 cm) activity cages. Gonads were regressed in all birds prior to the experiment as determined by unilateral laparotomy. All birds were placed on continuous light (LL) two days prior to the start of drug injections and maintained on LL during the 14 days of treatment. One group consisted of five males and one female. Each bird in this group received a daily injection (sc) of corticosterone followed in 11 h by an injection of DOPA (CS: 11, DOPA). A second group of five males and two females received daily injections of 5-HTP followed in 11 h by DOPA. To restrict further the timing of serotonin synthesis, PCPA (Appendix 1), a long-lasting inhibitor of serotonin synthesis, was injected daily 4 h after the DOPA injections (5-HTP: 11, DOPA: 4, PCPA). A third group of four males was maintained in continuous light for two weeks but received no injections.

After two weeks of injections the gonads were reexamined by laparotomy and the birds were transferred to a photoperiodic regimen (LD 16:8). During the ensuing months, observations of fat stores (see Section III) and molt indices were made biweekly. Molt was measured on a scale of one

(few feathers molting) to three (many feathers molting) for 10 body areas. Normal replacement of wing and tail flight feathers occurs simultaneously for opposite pairs and thus was recorded in these experiments only under this condition. Nocturnal migrants in cages also display restlessness at night which also has been shown to be a reliable indicator of a bird's physiological readiness to migrate (Weise, 1956). This Zugunruhe, or 'urge' to migrate was measured by keeping all birds in special activity cages and monitoring gross locomotor activity on Esterline-Angus event recorders. When a bird hopped to either of two perches, a microswitch circuit closed, and a mark was recorded on a moving chart. To quantify the locomotor activity for each bird, I counted the number of 9 minute intervals in which one or more pen deflections were recorded. This number was then converted into a percentage of the total number of 9-minute intervals on a given night. Thus a night of 8 hours would have 8 times 7 or 56 intervals during which activity could take place. If the bird had shown activity in 28 intervals, its activity would be 50 percent of the possible maximum.

Orientation was tested in Emlen funnels (Emlen and Emlen, 1966). An orientation cage consisted of a sheet of blotting paper formed into a cone and fitted into an aluminum pan so that the sides slope outward from the bottom at about 45 degrees. A thin ink pad is placed in the bottom of the pan after which the cone is covered with one-half inch

hardware cloth. Horizon glow was minimized by attaching a three inch high aluminum rim above the hardware cloth around the perimeter of each cage. Approximately 140 degrees of the sky is visible to a bird as it hops onto the sloping sides. A bird showing Zugunruhe behavior hops at frequent intervals from the ink pad onto the side of the cone and leaves a footprint record that provides an estimate of the bird's intended direction of flight. Orientation tests were conducted on the roof of a building at Louisiana State University on clear, moonless nights between 2100 and 2400 hours. Each funnel was divided into 16 equal sectors of 22.5 degrees each. The intensity of inky footprints in each segment was scored by comparison with a standard arbitrary scale of 1 (no footprints) to 20 (heaviest activity seen). Thus, a preferred direction is established by the concentration of higher values in a particular compass direction.

1978 Experiments

Because of the limited scope of treatment in the 1977 experiments and the inability to follow untreated control birds (see results, this section), a more extensive set of experiments was performed in October 1978. The white-throated sparrows were captured the previous winter and housed in an outdoor aviary. On October 19, two experimental groups were formed, one with males and the second with females. Unilateral laparotomies were performed to verify

regressed gonadal conditions prior to the experiment. Birds in both groups were exposed to continuous light during the injection period of October 20 to November 2 to avoid photoperiodic entrainment. Injections (sc) were made daily in each treatment regimen. The male sparrows were divided into four experimental groups as follows: 1) 5-HTP followed 12 h later by DOPA and PCPA (5-HTP: 12, DOPA-PCPA); 2) 5-HTP followed 12 h later by saline and PCPA (5-HTP: 12, saline-PCPA); 3) PCPA only; and 4) light only. Female sparrows were divided into six groups. Four of these groups received the same treatment as the male groups. Two additional groups received either 5-HTP followed in 8 h by DOPA and PCPA (5-HTP: 8, DOPA-PCPA) or 5-HTP followed 12 h later by DOPS (Appendix 1) and PCPA (5-HTP: 12, DOPS-PCPA). After two weeks of injections all birds were placed on a photoperiod regimen (LD 16:8). Each was examined periodically for gonadal development, fat, molt, nocturnal activity, and orientation as explained previously.

RESULTS

1977 Experiments

Gonadal growth occurred in at least some birds of both injection groups following the treatment period whereas none of the birds exposed to LL only showed any significant change from the regressed condition. Significant gonadal growth was apparent in 2 of the 6 (1 male, Table 1; and 1 female) in the CS: 11, DOPA group and in 5 of the 7 (3 males, Table 1; and 2 females) in the 5-HTP: 11, DOPA: 4, PCPA group. Maximal gonadal growth occurred approximately four weeks post-injection period. The period of gonadal development (6-8 weeks) approximated that observed in white-throated sparrows during the spring reproductive season. No change from the regressed gonadal condition was observed during the subsequent weeks of observation in any bird that did not respond to the initial treatment. Unfortunately, the four birds exposed to LL only died unaccountably shortly after the treatment period. Thus, their progress could not be compared with that of birds in the two experimental groups.

Fat stores of all birds in the two injection groups increased following treatment and peaked four to six weeks post-injection (Table 1). Fat levels dropped to near zero levels in both groups at 17 weeks post injection when a complete molt occurred in all birds. A rise in fat stores began about week 23 and coincided with the termination of molt and a reappearance of nocturnal activity (fig. 10).

All five birds (4 males, 1 female) remaining in the 5-HTP: 11, DOPA: 4, PCPA group exhibited distinct (active at least 33 percent per night) nocturnal activity at some time between weeks 0 and 13 post-injection. The orientation of this activity was northerly for the three birds in this group tested over 15 bird-nights (Fig. 11, Fall 1977). Five of six sparrows remaining in the CS: 11, DOPA group displayed significant nocturnal activity during the same time period. Orientation data is not available for this group. A second period of nocturnal activity was expressed by birds in both groups beginning at 21 weeks post-injection (Fig. 11, Spring 1978). The orientation of one bird (no. 874) tested from the CS: 11, DOPA group was directed southward over four test nights (Fig. 11). Two sparrows (nos. 891 and 893) from the 5-HTP: 11, DOPA: 4, PCPA group also oriented southward over nine bird-nights (Fig. 11).

All birds in both groups underwent a complete molt of contour and flight feathers at approximately 11 weeks post-injection although some birds replaced only a few pairs of flight feathers. Molt onset coincided with periods of low body fat stores, regressed gonads and absence of nocturnal activity. The molt in this sequence was typical of the complete postnuptial molt found in white-throated sparrows under natural conditions.

1978 Experiments

After the injection period, both testes and ovaries re-

mained regressed in birds that received only continuous light. However, varying frequencies and intensities of gonadal recrudescence occurred in all experimental groups. The highest frequency occurred in birds which received 5-HTP: 12, DOPA-PCPA. Nine of 10 males in this group had some testicular growth and five of seven females had at least one follicle greater than 1.5 mm in daimeter. At two weeks post-injection, recrudescence peaked for 7 of 10 males (Table 2). The response to the drug regimen was less variable and more quantifiable for males; left testis volumes ranged from 2 mm^3 (fully regressed) to 96 mm^3. The frequency of gonadal recrudescence was much less in other groups. Three of six males (Table 2) and two of six females in the 12-h saline group (5-HTP: 12, saline-PCPA) showed initial gonadal growth. At two weeks post-injection though, recrudescence was still observable in only two of six males (55 and 86 mm^3). Four others were fully regressed. The testes of all males in the saline group were regressed by six weeks post-injection. A similar pattern occurred in females of the saline group. One of six birds had significant follicle growth at two weeks post-injection and at four weeks all follicles were regressed.

When DOPA and PCPA were given eight hours after 5-HTP (5-HTP: 8, DOPA-PCPA) follicle growth was present in only one of five females (Fig. 12). The largest follicle in that bird increased from 1.5 mm in diameter at the end of injections to 2.2 at two weeks post-injection. At four weeks,

considerable ovarian development was present at zero and two weeks post-injection in two of six females that received DOPS and PCPA 12 hours after 5-HTP (5-HTP: 12, DOPS-PCPA) (Fig. 12). PCPA injected alone stimulated gonadal growth in one of seven males (Table 2) and in two of five females (Fig. 12).

In general, the patterns of administration of drugs that stimulated gonadal growth also favored fattening. The results with males were most distinct. Birds in the 5-HTP: 12, DOPA-PCPA group had significantly greater ($p \leq .05$, Student's t-test) subcutaneous fat stores than birds in either the 5-HTP: 12, saline-PCPA or LL only groups. The differences between these groups occurred from weeks two through six post-injection, a period which coincided with the period of gonadal recrudescence (Fig. 13). In contrast to the 1977 experiments, an initial fattening response was generally seen only in those birds which showed gonadal recrudescence (Table 1 vs. Table 2). Because elevated fat stores were present before injections, the possibility of a fattening response in the PCPA group is less clear. However, the one male in that group (no. 180) in which testis growth occurred had increased fat stores compared with other birds in that group at zero and two weeks post-injection (Table 2).

A second peak of fattening occurred in all 5-HTP: 12, DOPA-PCPA treated birds at 28 weeks after the end of in-

jections (Fig. 13) including three birds (nos. 197, 622, 633) which had little or no initial gonadal or fattening response (Table 2). A fattening response pattern is less certain in other groups at this time, partly because several birds had died in the interim. Three birds remained in the 5-HTP: 12, saline-PCPA group. A moderate increase in fat stores occurred in those two birds (nos. 714 and 172) which showed gonadal growth initially. Fat stores were very low in a third bird (no. 193). Fat stores reached near maximum levels for the three males which remained in the LL only group (Table 2).

All males monitored for locomotor activity (8 birds) in the 5-HTP: 12, DOPA-PCPA group exhibited a distinct period of nocturnal activity approximately between 0-8 post-injection. Intense nocturnal activity (> 50 percent possible per night) occurred in 6 birds as late as 15 December 1978 (week 8). Activity was monitored for three pairs of males in the 5-HTP: 12, saline-PCPA group. In general, a period of nocturnal activity was apparent for each pair until late November (weeks 0-5). However, intense activity (> 50 percent) ceased during approximately week 3. In general, fewer nights were conducive for orientation testing in 1978 than in 1977 during the autumn. As a result, no definitive orientation was evident in the 5-HTP: 12, DOPA-PCPA group.

Orientation in the 5-HTP: 12, saline-PCPA group over four bird-nights was directed slightly northeast (42^{o}) and

that of birds in the LL only group towards the southeast (127°) (Fig. 14, Fall 1978).

Orientation was tested again beginning approximately 27 weeks post-injection and coincided with the second period of nocturnal activity and fattening mentioned previously. Orientation of birds in the 5-HTP: 12, DOPA-PCPA group was more evident at this time and directed southwards (173°) over nine bird-nights (four different birds) (Fig. 14, Spring, 1977). One bird (no. 172) in the 5-HTP: 12, saline-PCPA group oriented west-southwest (248°) on two test nights. This bird had exhibited gonadal growth originally after the initial injection period (Table 2). Pronounced noctural activity occurred in three males remaining from the LL group at this time. Orientation was tested for each over five nights. The orientation for two was southwesterly (217° and 245°), but that for the third was directed to the north-northeast (32°). Apparently, the orientation of at least two did not deviate markedly from the mean orientation (127°) that occurred when tested shortly after the treatment period.

A period of molt in varying degrees occurred in birds of all groups. In males, an intense complete molt of body and flight feathers was apparent in 9 of 10 in the 5-HTP: 12, DOPA-PCPA group and in all six birds in the 5-HTP: 12, saline-PCPA group (Fig. 13 A and B). In the PCPA group, 1 of 7 males had a complete molt (Fig. 13 C). That particular bird, no. 180 (Table 2), was the only one that showed some

gonadal recrudescence. The remaining six birds molted body feathers only and not flight feathers at the same time. All six males that had received continuous light only had a partial molt of body feathers but there was not replacement of flight feathers which is a characteristic of the post-nuptial molt. The molt of body feathers occurred at the same time as that in other groups.

Table 1

Left testis volume (mm^3) and fat index in white-throated sparrows (Zonotrichia albicollis) subsequent to a period of daily injections during October, 1977 of corticosterone or monoamine-affecting drugs in specific hourly relations.

	Weeks post-injection period							
Corticosterone: 11, DOPA[a]								
Bird	Pre	0	2	4	6	8	10	13
838	2/10[c]	2/18	2/23	2/25	2/20	2/20	2/23	2/20
878	2/15	6/25	2/23	2/18	2/23	2/8	2/8	2/5
874	2/13	2/18	2/18	2/23	2/23	2/23	2/15	2/10
896	2/10	2/18	2/35	2/45	2/40	2/35	2/35	2/5
895	4/10	25/13	62/25	68/23	9/35	3/20	2/23	2/3
5-HTP: 11, DOPA: 4, PCPA[b]								
893	2/25	2/15	2/35	2/25	2/23	2/10	2/10	2/10
892	2/8	22/18	37/20	62/23	61/30	26/23	6/10	2/10
883	2/18	5/8	2/10	2/38	2/30	2/23	2/10	2/5
891	2/15	13/10	57/25	54/30	58/45	27/40	8/35	2/18
888	3/10	47/20	--	--	--	--	--	--
Continuous light only								
573	2/18	4/5						
732	2/5	2/3						
873	2/3	2/8						
640	2/18	2/15						

[a]Daily injections of corticosterone followed in 11 hours by DOPA for 14 days.

[b]Daily injections of 5-HTP followed in 11 hours by DOPA followed in 4 hours by PCPA for 14 days.

[c]Left testis volume (first numeral of 'fraction') and fat index (range: 0 - 40). Testis volumes two (2) mm^3 or less are designated '2'.

Figure 10. Effects of corticosterone or serotonin and catecholamine-affecting drugs on the patterns of gonadal development, fat stores, molt and nocturnal activity in white-throated sparrows in autumn condition. Photorefractory birds were placed in continuous light in October, 1977 and injected daily for 14 days with 5-HTP followed in 11 hours by DOPA followed 4 hours later by PCPA (A) or corticosterone followed in 11 hours by DOPA (B). Following injections the birds were maintained on LD 16:8. Testes were regressed (2 mm^3) in four birds before and after continuous light treatment alone. Post-injection examinations were generally biweekly. Gonadal growth (closed circles) and fat stores (open circles) were measured as before (see legend, Fig. 6). Dashed lines denote projected changes during the injection period. Closed bars are periods of complete molt of body and flight feathers. The adjacent fractions represent the proportion of birds in molt. Open bars are periods of nocturnal activity. Orientational preference as indicated by N (northwards) or S (southwards) was determined in Emlen cages under the open, night sky.

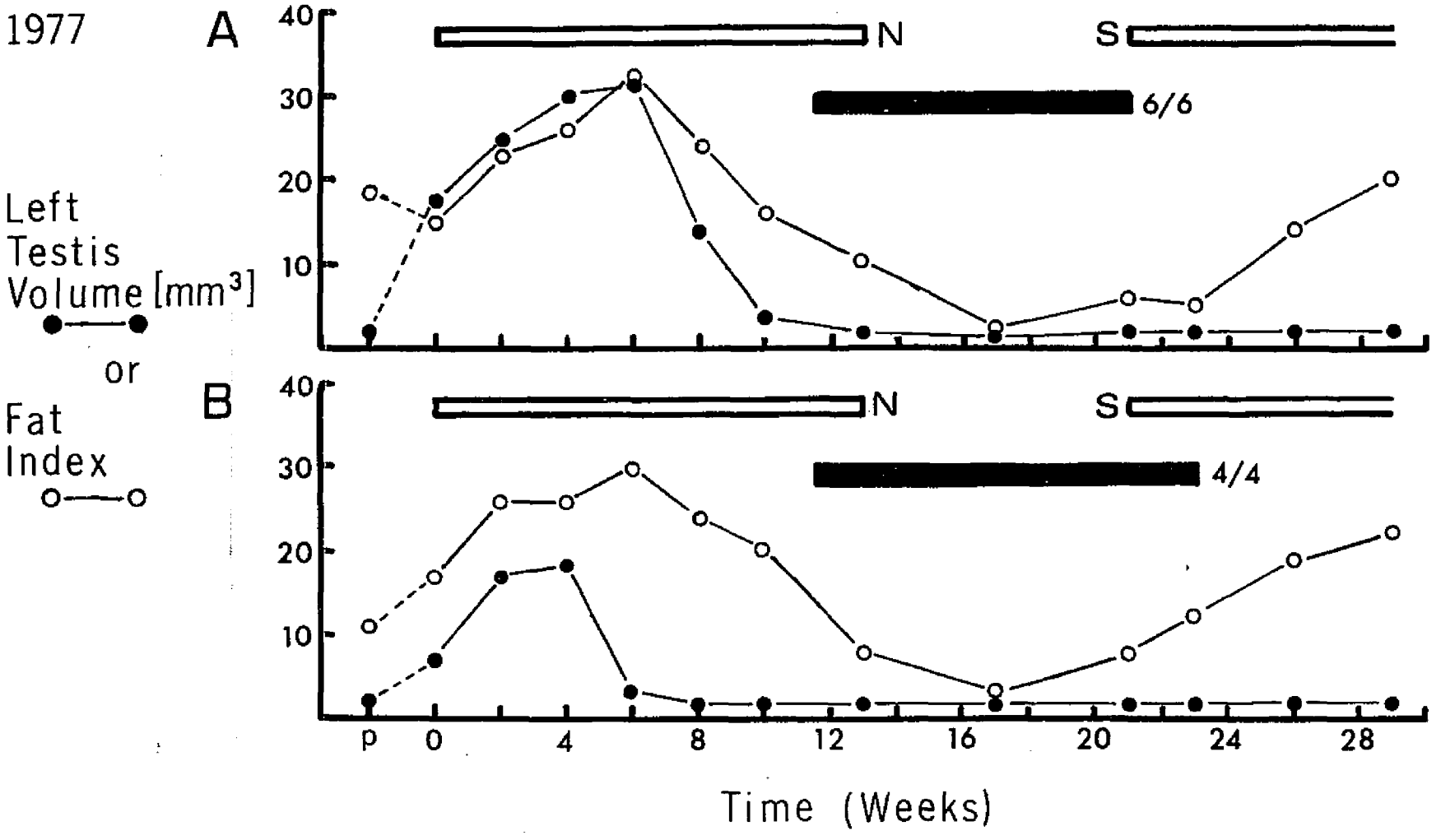

1977
A
B
Left
Testis
Volume [mm³]
or
Fat
Index
40
30
20
10
N
S
6/6
4/4
p
0
4
8
12
16
20
24
28
Time (Weeks)

Figure 11. Orientation of white-throated sparrows tested at two seasons subsequent to a 14-day period (October, 1977) of daily injections of DOPA 11 h after either 5-HTP or corticosterone. Orientation during the first period of nocturnal activity (Fall 1977) was tested during November 1977 in three birds injected with the 5-HTP: DOPA regimen. Orientation during the second period of nocturnal activity (Spring 1978) was tested during April 1978 in two 5-HTP: DOPA-injected birds (891, 893) and one corticosterone: DOPA-injected bird (874). Diagrams are plotted such that the radius equals the greatest number of activity units in any sector totalled for all test nights for a particular bird. This value is found at the perimeter of each diagram and represents the mean direction of all nightly activity. Triangles represent nightly mean headings.

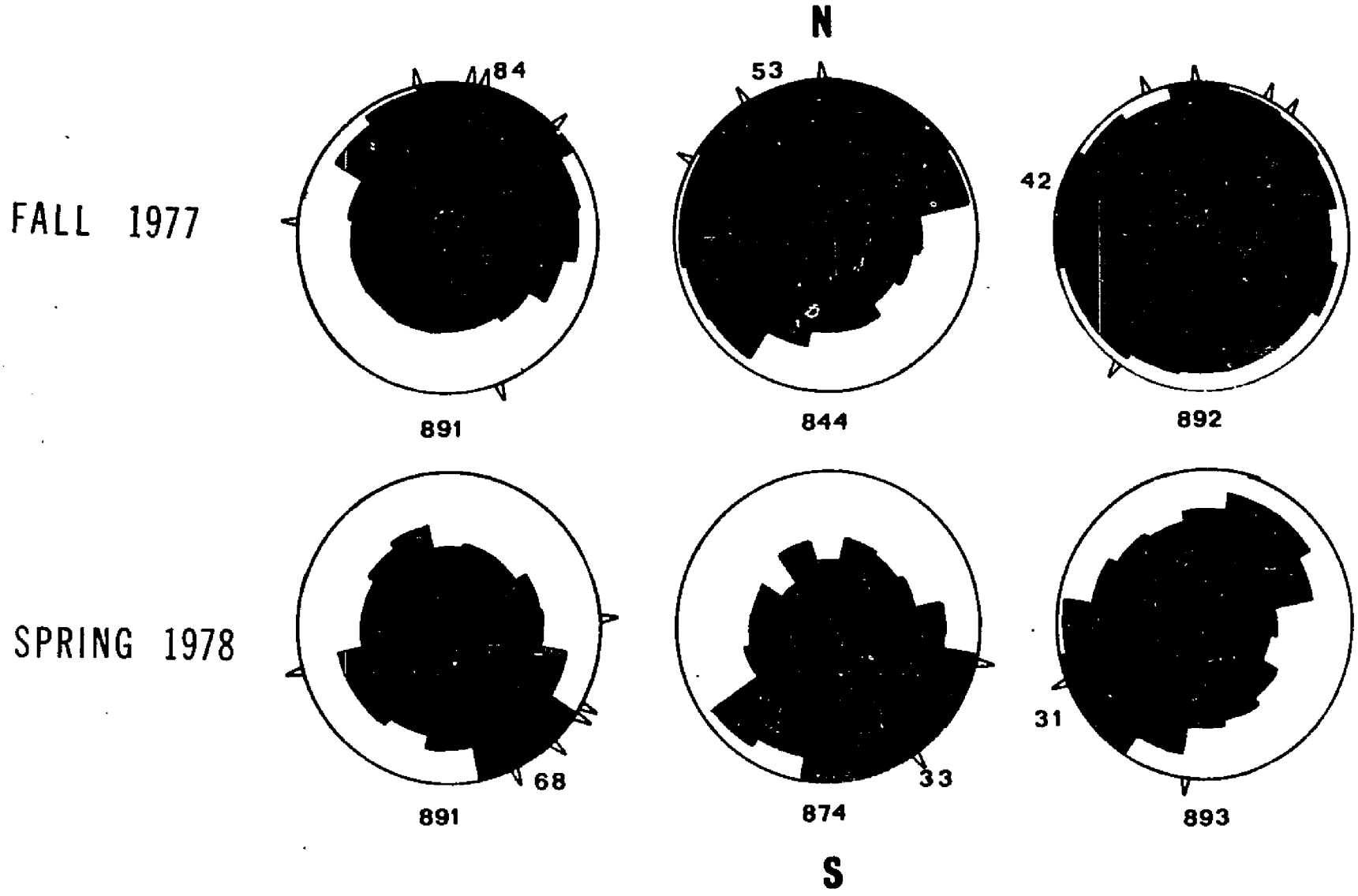

N
FALL 1977
84
53
42
891
844
892
SPRING 1978
68
33
31
891
874
893
S

Table 2

Left testis volume (mm^3) and fat index in white-throated sparrows (Zonotrichia albicollis) subsequent to a period of daily injections of monamine-affecting drugs in specific hourly relations.

		Weeks post-injection period						
5-HTP: 12, DOPA-PCPA[a]								
Bird	Pre	0	2	4	6	8.....24		28
623	2/5[c]	5/13	51/20	4/13	2/10	2/3	2/33	2/40
625	2/3	36/20	27/5	4/3	3/5	2/5	--	--
197	2/10	12/3	2/25	2/10	2/13	2/3	2/10	2/40
604	2/3	23/25	64/40	17/40	2/33	2/5	2/35	--
631	2/8	27/23	46/28	5/23	2/28	2/5	2/3	2/40
608	2/5	21/20	71/40	68/40	17/38	2/5	2/5	2/13
603	2/8	73/23	96/30	30/23	2/18	2/3	2/8	2/30
622	2/10	2/3	2/10	3/10	2/13	2/3	2/3	2/23
633	2/10	11/3	4/10	2/18	2/10	2/0	2/8	2/33
115	2/8	8/25	32/30	4/13	2/18	2/5	2/33	2/15
5-HTP: 12, saline-PCPA[b]								
Bird	Pre	0	2	4	6	8.....24		28
193	2/5	2/15	2/10	2/8	2/8	2/3	2/3	2/3
714	2/10	11/13	55/25	43/23	2/20	2/5	2/8	2/28
614	2/5	2/15	2/3	2/3	2/0	2/3	--	--
483	2/13	8/15	2/15	2/10	2/8	2/5	--	--
738	2/8	2/8	2/3	2/10	2/10	2/3	--	--
172	2/3	15/18	86/25	5/5	2/15	2/3	2/20	2/15
PCPA Only								
Bird	Pre	0	2	4	6	8.....24		28
180	2/25	34/30	31/23	2/3	2/8	2/13	--	--
N = 6	2/22	2/17	2/5	2/5	2/5	2/10	2/18	2/19

Table 2 Continued

Continuous Light Only

Bird	Pre	0	2	4	6	8.....24		28
N = 6	2/18	2/23	2/12	2/9	2/9	2/12	2/40	2/30

[a]Daily injections of 5-HTP followed in 12 hours by DOPA and PCPA for 14 days.

[b]Daily injections of 5-HTP followed in 12 hours by saline and PCPA for 14 days.

[c]Left testis volume (first numeral of 'fraction') and fat index (range: 0 - 40). Testis volumes two (2) mm^3 or less are designated '2'.

Figure 12. Development of ovarian follicles in white-throated sparrows in autumn condition after treatment with serotonin and catecholamine-affecting drugs. The protocol was the same as that described in Figure 11. The numerals beside the arrows indicate the hourly interval between injections. Follicle growth was determined by laparotomy using fine forceps to estimate the diameter of the largest follicle seen. The number of birds in each group is given within bars.

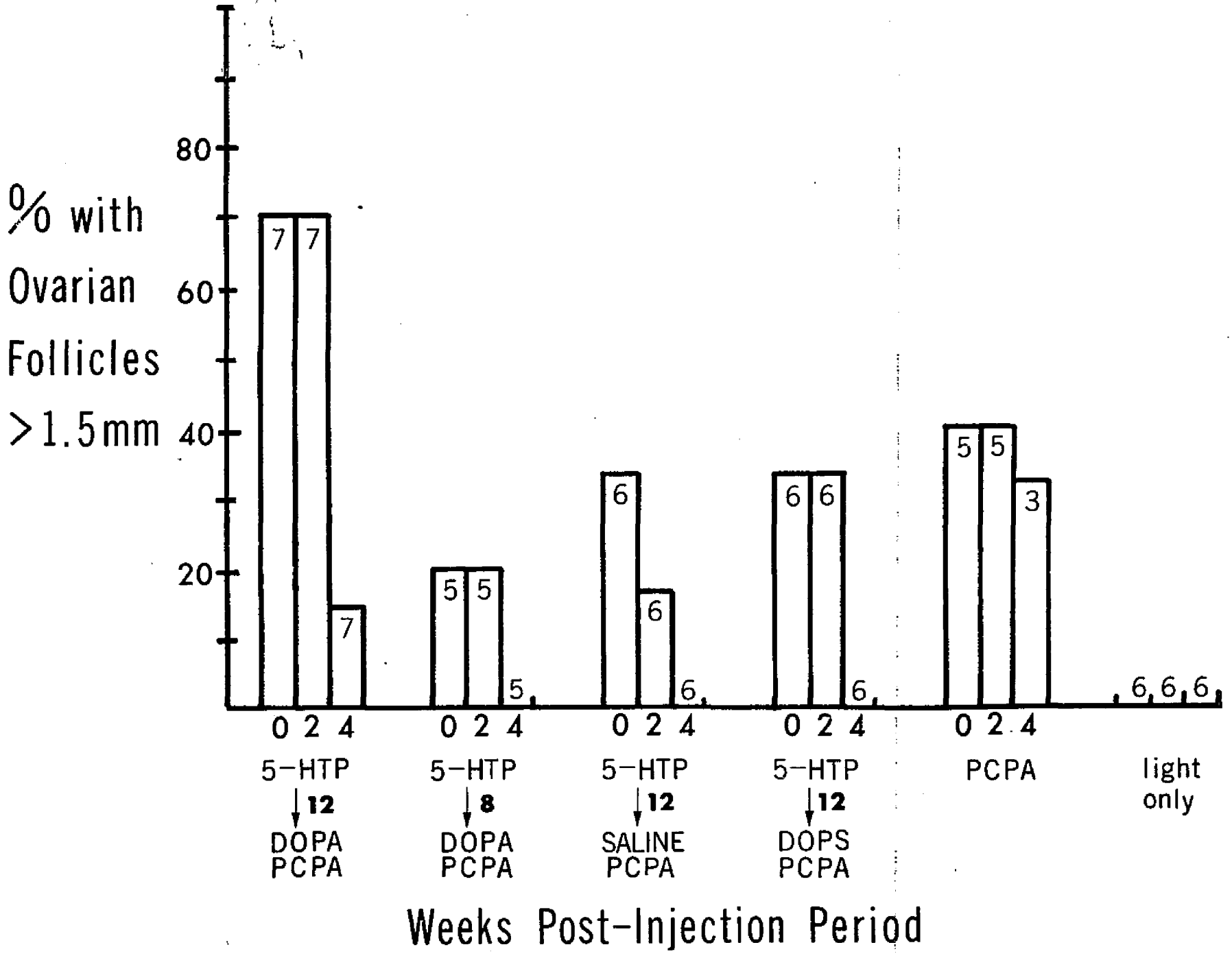

% with Ovarian Follicles >1.5mm
80
60
40
20
7 7 7
5 5 5
6 6 6
6 6 6
5 5 3
6 6 6
0 2 4
5-HTP
12
DOPA PCPA
5-HTP
8
DOPA PCPA
5-HTP
12
SALINE PCPA
5-HTP
12
DOPS PCPA
PCPA
light only
Weeks Post-Injection Period

Figure 13. Effects of serotonin and catecholamine-affecting drugs on gonadal development, fat stores, molt, and nocturnal activity in white-throated sparrows treated in 1978.

Photorefractory birds were placed in continuous light in October, 1978 and injected daily for 14 days: (A) 5-HTP followed in 12 hours by DOPA and PCPA. (B) 5-HTP followed in 12 hours by saline and PCPA. (C) PCPA alone. (D) Continuous light only. Measurements for the various indices and symbols used are as described previously (Figure 10). Orientation was not tested during the second period of nocturnal activity in group (B) and was equivocal in group (D).

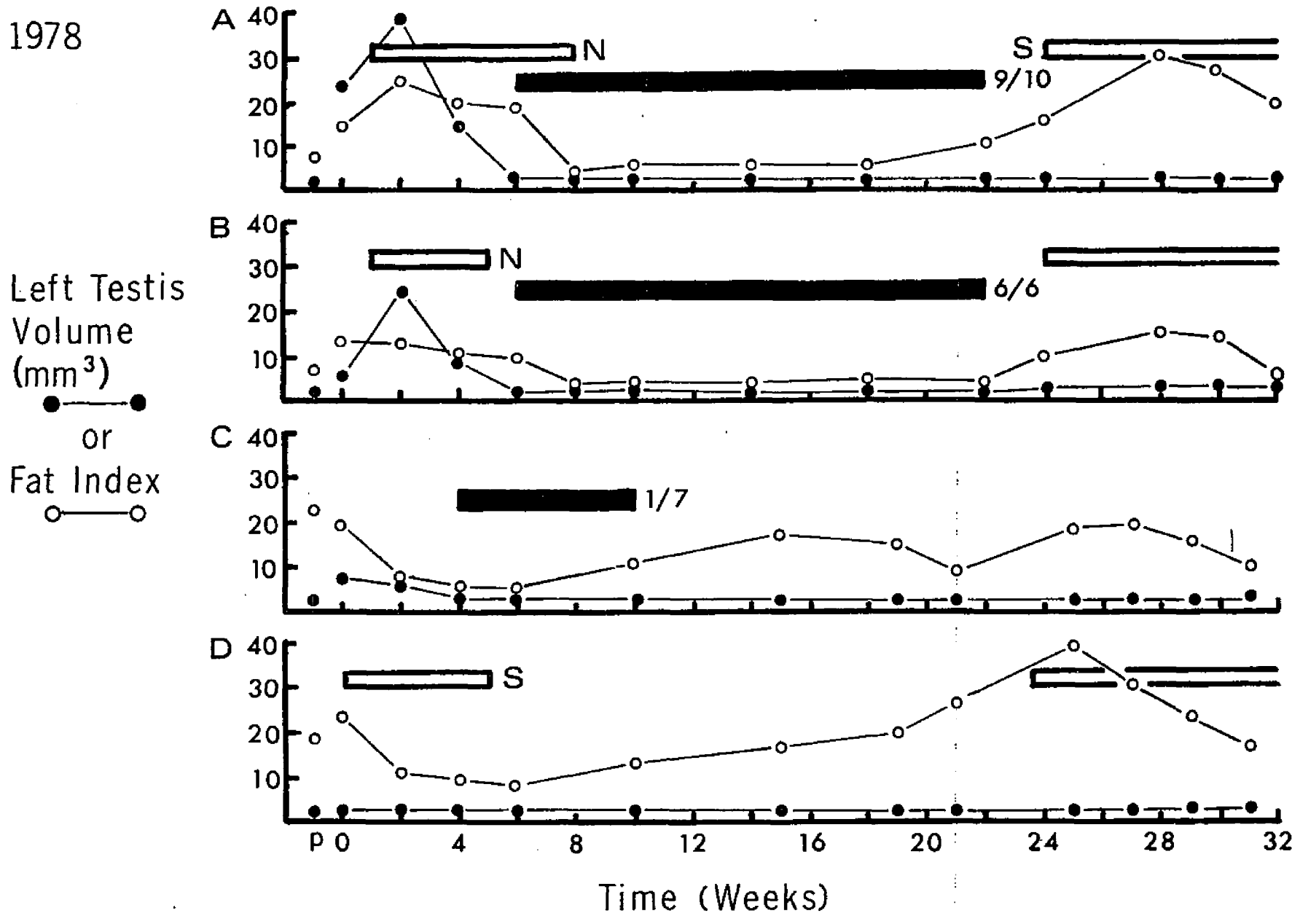
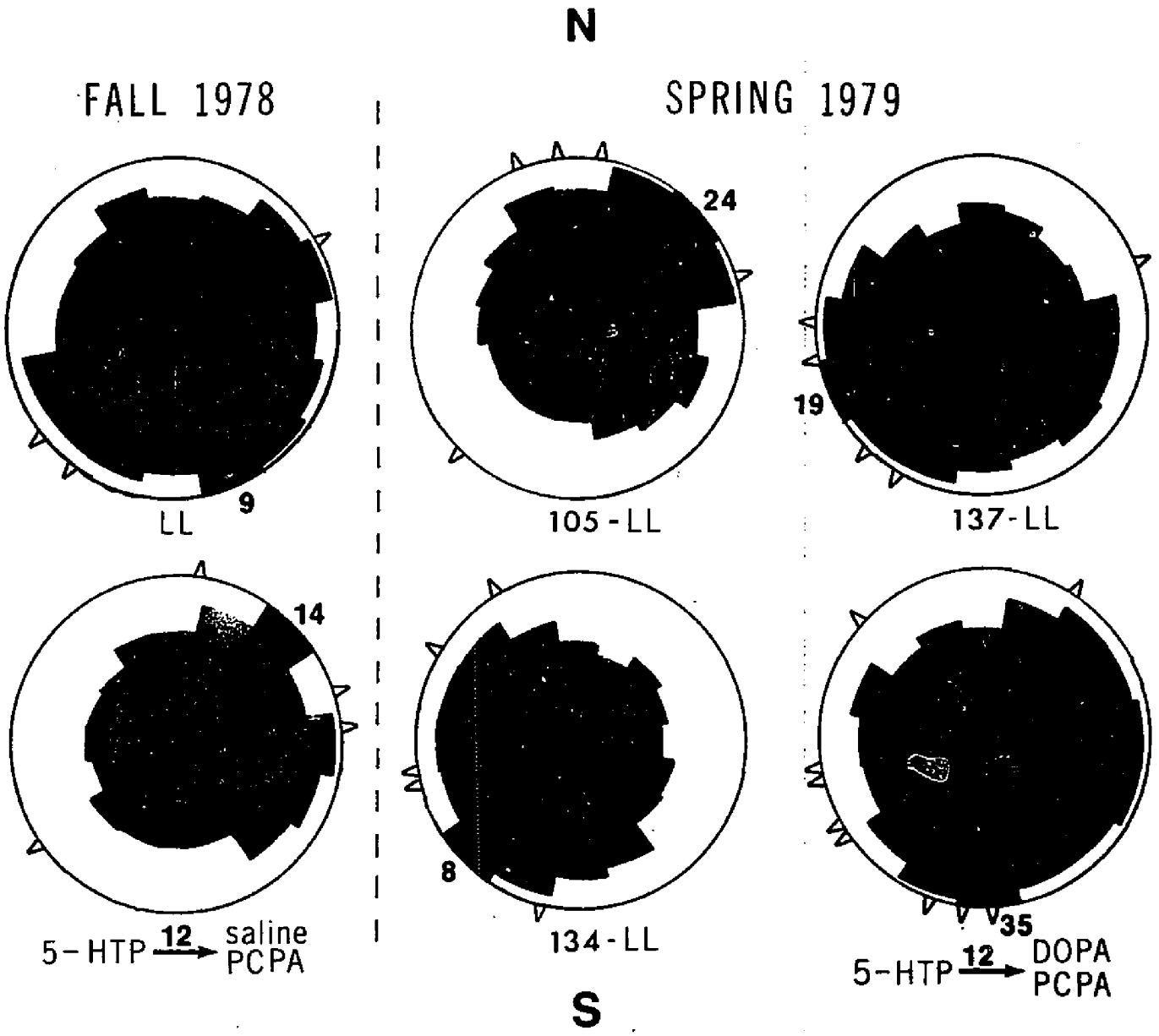

1978
A
B
C
D
40
30
20
10
N
S
9/10
6/6
1/7
Left Testis Volume (mm³)
or
Fat Index
P 0
4
8
12
16
20
24
28
32
Time (Weeks)

Figure 14. Orientation of white-throated sparrows tested at two seasons subsequent to a 14-day period of daily drug injections during October, 1978. Orientation during the first period of nocturnal activity (Fall 1978) was tested in birds exposed to continuous light (LL) only during the injection period and in birds injected with saline and PCPA 12 h after 5-HTP (5-HTP: 12, saline-PCPA). Orientation during the second period of nocturnal activity (Spring 1979) is shown for birds originally injected with DOPA and PCPA 12 h after 5-HTP (5-HTP: 12, DOPA-PCPA) and for three individuals (105, 137, 134) exposed to LL only. Diagrams are explained in Figure 11.


N
FALL 1978
SPRING 1979
9
LL
24
105 - LL
19
137 - LL
14
5 - HTP 12 saline PCPA
8
134 - LL
35
5 - HTP 12 DOPA PCPA
S

DISCUSSION

The experiments reported here on white-throated sparrows were performed to test whether drugs which alter particular neurotransmitter activities can reset the circannual cycle. The results of experiments with photosensitive sparrows in March and June (Section III) indicated that daily injections of DOPA at 3, 8, or 18 hours after 5-HTP injections inhibited the normal photoperiodic increase in gonadal growth whereas a 12-h injection interval was stimulatory for these conditions. Injections of prolactin 12 h after corticosterone also stimulated spring conditions (Meier and Martin, 1971; Meier et al, 1971b; Martin and Meier, 1973; Ferrell, 1979). In the present experiments (Section IV), the 12-h relation of 5-HTP and DOPA appears to have reset the annual cycle from fall conditions to spring conditions.

The results of the 1977 experiments indicate that injections of DOPA 11 to 12 hours after either corticosterone or 5-HTP were capable of resetting autumn sparrows into the spring condition. At least some birds in both groups displayed the entire range of conditions which occur during the normal annual cycle. Sparrows which did not exhibit gonadal recrudescence nonetheless displayed other features such as a fat cycle, molt, and nocturnal activity at the appropriate times. The group which received 5-HTP was more successful in stimulating all features of the spring condition than the

group which received corticosterone. Serotonergic stimulation by 5-HTP injection seems to be more effective than corticosterone in stimulating those physiological processes which cause the release of gonadotropins.

Since the four uninjected birds in 1977 died shortly after the end of the injection period, their long-term response to a 14-day interval of continuous light and then 16-h daylengths is unknown. However, no bird showed any observable change in testis size after two weeks of continuous light. The limited data indicate that the reproductive system was not altered from a photorefractory condition. Shank (1959) and Wolfson (1958) carefully studied the refractory period in white-throated sparrows and found that this species does not become photosensitive until the early part of December. Thus the stimulatory effects seen in the experimental groups are unlikely to be due to photostimulation alone in October.

The data accumulated in 1978 also show a differential response to drug treatment and their temporal administration. The 12-h treatment interval (5-HTP: 12, DOPA-PCPA) was clearly more stimulatory for ovarian development than the 8-h interval. A salient feature of these experiments is that at least some reproductive growth occurred in individuals of every group except those which were exposed to light only. The only drug common to all injection regimens was PCPA. The rationale for its administration was that it might limit

serotonergic activity to an interval following 5-HTP injection since PCPA is a potent inhibitor of the conversion of tryptophan to 5-HTP. A single injection to rats in a dosage used in this experiment reportedly inhibited tryptophan hydroxylase for up to 96 hours (Koe and Weismann, 1966). PCPA was given once per day in treatments reported here. Therefore, endogenous serotonin should have been low over a 2-week injection period in those birds which did not receive 5-HTP. This effect was apparently sufficient to stimulate some gonadal recrudescence in one male and two females out of 12 birds. Apparently, PCPA alone reset the annual cycle in these birds since they experienced a complete post-nuptial molt when the gonads regressed again after several weeks. Thus, the photorefractory condition may be maintained in part by high serotonin levels. Serotonin in this role may be acting as a synaptic modulator to promote or inhibit the passage of particular neural stimuli (review: Myers, 1974). However, the improved response of birds which received injections of catecholamine and serotonin precursors indicates that seasonal changes in brain serotonin levels is an inadequate explanation for seasonal refractoriness and sensitivity and further suggests that a synergism of circadian neural oscillators is involved in circannual cycles. 5-HTP and DOPA in the 12-h relation was most successful in stimulating the entire range of annual conditions in both male and female groups.

When saline was substituted for DOPA, gonadal responsiveness dropped markedly. Furthermore, the lack of responsiveness when DOPS was substituted for DOPA suggests that dopamine, and not noradrenaline, is the catecholamine that is preferentially involved. DOPS is a specific precursor of noradrenaline, but does not necessarily affect dopamine synthesis from its amino acid precursor, tyrosine. Thus, without specific inhibition of dopamine production concurrent with stimulation of noradrenaline, the ovarian development of 2 of 6 females in the 5-HTP: 12, DOPS-PCPA group could have been due to the continued presence of dopaminergic activity. When a more selective treatment (inhibition of noradrenaline synthesis with DDC) was done with spring photosensitive sparrows (Section III, (Exp. 2), the data support a hypothesis for the specific role of dopamine, not noradrenaline, as one of the synergistic components in the regulation of the annual cycle.

Increased fat stores are associated in migrants with periods of migratory activity. In the spring, gonadal recrudescence also accompanies elevated fat levels as the birds approach their breeding grounds. The vernal conditions appear following the 5-HTP: 12, DOPA-PCPA treatment and the heaviest fat stores found in this study were in that group. Heavy fat stores are also induced by a 12-h corticosterone-prolactin injection regimen (Meier and Martin, 1971; Meier

et al., 1971b). Curiously, initial fattening was more closely correlated with gonadal growth in 1978 than in 1977. Birds without a gonadal response in 1977 still had increased fat stores following injections. However, even those three males (5-HTP: 12, DOPA-PCPA group) which had little or no initial gonadal or fattening response in 1978 showed an increase in fat stores with other birds at week 28 post-injection (Table 2).

The occurrence of nocturnal activity and northward orientation during the post-injection period support other data which indicates that drug injections had reset birds into a spring migratory condition. In contrast, the southeasterly orientation of LL control birds suggests that normal autumn migratory activity continued during and after the injection period. Subsequent southward orientation in the drug-treated groups after passage of spring and summer conditions indicates that birds had entered a fall migratory condition according to the natural sequence. Interestingly, three remaining LL control males also became active during the second activity period (week 27) of the experimental groups. Two oriented approximately southwest but the third oriented strongly to the northeast. This dichotomous behavior makes assessment of their seasonal state difficult. Heavy fat stores without accompanying gonadal growth suggests the presence of a recurring fall condition. A period of short days is probably necessary to 'break' fall conditions.

However, no definite conclusions should be drawn from these limited data.

A further consideration is that not all treated birds exhibited the entire range of seasonal conditions. One male in the 5-HTP: 12, DOPA-PCPA group which had pronounced gonadal growth failed to molt flight feathers although it did molt body feathers heavily. Conversely, four males in the 5-HTP; 12, saline-PCPA group which showed little or no gonadal growth still displayed a complete molt with flight feather replacement. A similar pattern occurred with photorefractory white-throated sparrows injected with a 12-h relation of corticosterone and prolactin in July or August (Ferrell, 1979). Although the injections stimulated the full range of spring conditions in most birds, a few exhibited no gonadal growth. Nonetheless, those birds later had a complete molt of flight feathers at the same time as the others. Apparently, the injections had reset their annual cycle as well, in spite of the initial lack of gonadal growth. The neural components which synchronize molt to other physiological conditions may become 'uncoupled' from the principal neural oscillation. Other studies with Zonotrichia have shown an independence of molt from other features of the annual cycle under experimental conditions (Wolfson, 1954; Weise, 1962; Sansum and King, 1976; Miller and Weise, 1978).

These experiments support the hypothesis that regulation of the annual cycle involves two circadian neural oscillations. Serotonergic activity (maintains corticosterone rhythm) and dopaminergic activity (regulates prolactin release) appear to be expressions of these oscillations. Timed daily injections of either corticosterone and prolactin (Ferrell, 1979) or 5-HTP and DOPA apparently reentrain these oscillations into the spring pattern when given at 12-h intervals. The daily rhythms of corticosterone and prolactin shift in phase with each other during the year (Meier and MacGregor, 1972). Neurotransmitter activity associated with these rhythms must also vary during the day and throughout the year. This change in phase relationship is believed to reflect the annual phase shift that occurs between the two neural oscillators. Thus, a temporal interaction between two circadian rhythms which change in phase with each other throughout the year may account for the induction of the different metabolic and behavioral events in the annual cycle.

SUMMARY

A single injection of 5-HTP (serotonin precursor) raised plasma corticosterone concentration in Japanese quail and white-throated sparrows one to three hours after injection. Serotonergic activity may be involved in maintenance of the daily rhythm of plasma corticosterone. A period of daily 5-HTP injections induced gonadal growth in quail and house sparrows when given at certain times relative to a short daily photoperiod but not at other times, and indicates that timed serotonergic activity entrains a daily rhythm of photosensitivity.

In white-throated sparrows, DOPA (catecholamine precursor) induced a variation in gonadal growth and fattening depending on the time of daily injection relative to a 5-HTP injection. A 3, 8, or 12-h interval of drug injections produced responses which approximated conditions seen during the fall migratory, summer refractory, and spring migratory seasons, respectively. In house sparrows, daily injections of DOPA 16 h after 5-HTP depressed oviduct development whereas a 8-h interval induced no change from the photostimulated, pre-experimental condition.

A 14-day period of 5-HTP and DOPA injections in the 12-h relation apparently reset the circannual clock in photorefractory white-throated sparrows during the fall so that their annual cycle was reset to the spring condition. Spring

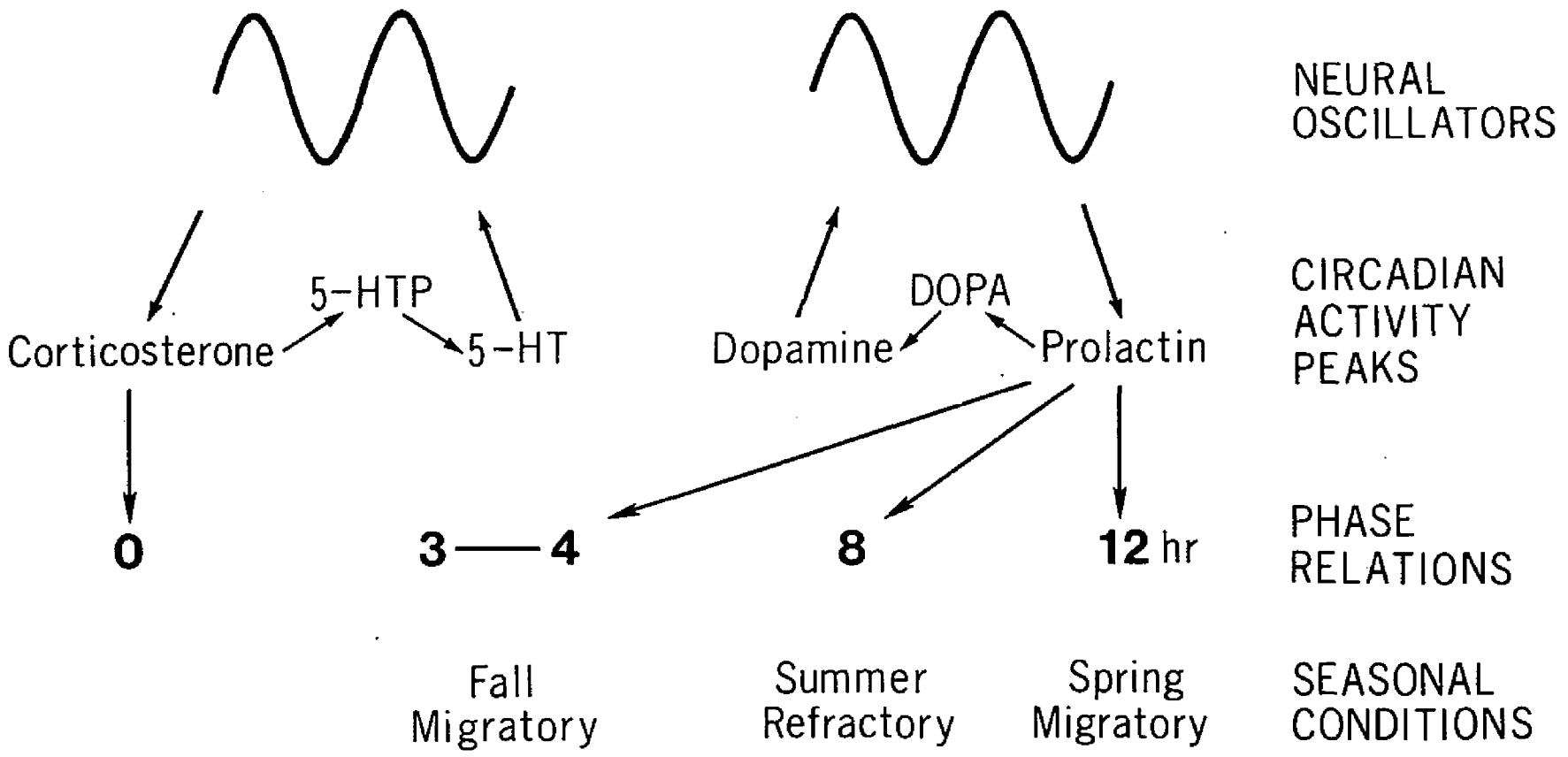
conditions subsequently were replaced by summer and fall conditions in a manner similar to the natural seasonal progression.

The results indicate that a temporal synergism between daily serotonergic and catecholaminergic activities affects gonadal development and fattening responses. Similar results were reported when daily corticosterone and prolactin injections were made in the same hourly relations. The present experiments support the hypotheses that: 1) a daily rise in corticosterone levels and serotonergic activity entrain a daily rhythm of tissue sensitivity and 2) the daily plasma prolactin rhythm in white-throated sparrows is regulated by catecholaminergic (possibly dopaminergic) activity. Daily hormone or drug injections in specific hourly intervals produce conditions which mimic different seasonal states. The injection time intervals are believed to reflect the intervals between the endogenous daily peaks of hormone and neurotransmitter activities that exist throughout the year. Both daily hormonal and neurotransmitter activities appear to be the metabolic expressions of two circadian neural oscillations.

I believe the results support the concept that the changing relations of at least two neural circadian oscillations regulate the annual cycle. I propose that daily serotonergic and catecholaminergic activities are elements

in this endogenous circannual mechanism. Figure 15 illustrates the hormonal and neuroendocrine relationships thought to occur. Future experiments will undoubtedly reveal additional elements involved in circannual regulation.

Figure 15: Model of the circadian mechanisms believed to regulate the circannual cycle of reproductive and migratory conditions in the white-throated sparrow. Two circadian neural oscillations control many daily events including the circadian rhythms of plasma corticosterone and prolactin concentrations. The temporal interaction of the two circadian oscillations and their circadian expressions determine the complex of metabolic and behavioral conditions appropriate for specific seasons. A circannual shift in the phase relations of the two circadian oscillations produces the orderly sequence of seasonal conditions. The phase relations of the two oscillations may be reset by timed daily injections of hormones (corticosterone and prolactin) and drugs which influence neurotransmitter (serotonin and dopamine) activities.


NEURAL OSCILLATORS
CIRCADIAN ACTIVITY PEAKS
PHASE RELATIONS
SEASONAL CONDITIONS
5-HTP
Corticosterone
5-HT
DOPA
Dopamine
Prolactin
0
3 — 4
8
12 hr
Fall Migratory
Summer Refractory
Spring Migratory

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APPENDIX 1

Serotonin and catecholamine-affecting drugs.

Type	Drug	Abbreviation	Dosage (mg/kg)	Action	Resulting Action	Reference
Serotonergic affecting	5-hydroxy-tryptophan	5-HTP	80	immediate precursor molecule to serotonin	raises serotonin titers	Ternaux et al., 1975
	p-chloro-phenyl-alanine	PCPA	120	inhibits tryptophan hydroxylase	lowers serotonin titers	Koe and Weismann, 1966
	Fluoxetine (Lilly 11040)	--	10	prevents reuptake of serotonin into axon terminal	potentiates serotonergic activity in synaptic cleft	Wong et al. 1974
Catecholaminergic affecting	L-dihydroxy-phenylalanine	L-DOPA	150	immediate precursor to dopamine	raises dopamine and noradrenaline titers	Shindo et al., 1973
	dihydroxy-phenyl-serine	DOPS	150	immediate precursor to noradrenaline	raises noradrenaline titers	El Halawani and Burke, 1975

Appendix 1 Continued

Drug	Abbreviation	Dosage (mg/kg)	Action	Resulting Action	Reference
Na-diethyl-dithio-carbamate	DDC	150	inhibits dopamine B-hydroxylase	raises dopamine, lowers noradrenaline titers	Carlsson et al., 1966
α-methyl para-tyrosine	α-MT	400	inhibits tyrosine hydroxylase	lowers catecholamine titers	Spector et al., 1964

VITA

Larry John Miller was born June 12, 1951, in Rochester, New York. He attended the State University of New York at Stony Brook where he received the Bachelor of Science degree in May, 1973. In June of that year he married Keiko Takioto. Mr. Miller entered graduate school in the Department of Zoology at the University of Wisconsin - Milwaukee in September, 1973. Under the direction of Dr. Charles M. Weise he received the Master of Science degree in May, 1976. Mr. Miller entered Louisiana State University in August, 1976 to pursue the degree of Doctor of Philosophy in the Department of Zoology and Physiology. He has been employed as an Instructor in the Department of Zoology and Physiology at Louisiana State University since August, 1979. He is also a candidate for the degree of Doctor of Philosophy at Louisiana State University.